Introduction

NCI Scientific Core Resource Directory

The following pages describe the extensive array of scientific resources and special expertise that is available to support NCI's Intramural research efforts. The facilities included in this directory offer such cutting-edge technologies as high-throughput screening, sequencing, gene expression profiling, and proteomics resources, as well as an expansive assortment of essential conventional services. Taken together, this vast collection of scientific expertise and technological resources provide Investigators of the NCI Intramural Research Program with one of the richest environments in the world to conduct basic or clinical research.

Each entry in the Directory includes a "mission statement" and available expertise in each core, as well as a detailed list of available technologies and those being developed or newly implemented. In addition, lists of collaborative efforts and recent publications are included to provide a sense of how intramural investigators are making use of these facilities.

Core facilities and resources are managed through a variety of support mechanisms, with the **Research Technology Program (RTP)**, located at the NCI-Frederick campus, prominent among them. The RTP was established by NCI's Office of the Director and is managed by Science Applications International Corporation (SAIC-Frederick, Inc.) and includes seven specialized technology centers as well as two support service centers. Novel technological resources are also made available through NCI's **Advanced Technology Center**, which houses investigators from NCI's Intramural Divisions and the National Human Genome Research Institute (NHGRI). Other examples include the Tissue Array Program, another collaborative effort between NCI and NHGRI. In addition, the **Center for Cancer Research** hosts several core resources to support its investigators.

The facilities in this report are generally listed as having "open" access - that is, available to any NCI researcher (usually on a fee-for-service basis). Other resources listed as "Lab/Program dedicated", are accessible on an "as available" basis, usually at the discretion of the Lab/Branch Chief or Program Manager. Such access usually involves a scientific collaboration. At the very least, these "dedicated" facilities provide a rich source for expert advice.

The NCI is committed to providing the best possible support structure. Please contact any of the editors with comments or suggestions to improve our resources.

Editors

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Special Assistant to the Director
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Mass Spectrometry Center
Analytical Chemistry Laboratory (ACL)

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Research Technology Program, SAIC-Frederick, Inc.

Web page: http://web.ncifcrf.gov/rtp/labs/ACL/ACL.asp

Detection, quantitation, structure confirmation and accurate mass measurements for biomolecules

Mission: We work collaboratively with NCI and other investigators to develop new technologies to further our understanding of cancer. The center will be equipped with state-of-the-art mass spectrometry instrumentation for the analysis of both small and large biomolecules. The center will also educate scientists on the use, interpretation, and applications of mass spectrometry.

Expertise: Drs. Timothy Veenstra and Thomas Conrads have recently been hired as the Director and Associate Director of the Mass Spectrometry Center, respectively. They are currently recruiting scientists to supplement specialties in the analysis of small and large biological molecules using a variety of MS-based technologies.

The center offers expertise in detection, quantitation, structure confirmation and mass measurements for small molecules, such as natural products, synthetic intermediates, metabolites, nucleosides, peptides and other endogenous molecules.

Established Technologies: The Mass Spectrometry Center offers

Electron ionization mass spectrometry

Chemical ionization mass spectrometry

Liquid secondary ion mass spectrometry

Matrix-assisted laser desorption (MALDI) mass spectrometry

GC and solid probe sample introduction methods.

The Mass Spectrometry Center is expanding its available expertise and equipment. This expansion will afford investigators the opportunities for the analysis and discovery of biomolecules involved in the expression and progression of cancer. The following equipment is expected to be installed and operational before January 2002:

Four LCQ Deca XP mass spectrometers – a highly sensitive ion-trap mass spectrometer ideal for the identification of proteins and peptides.

API QSTAR Pulsar – a hybrid quadrupole time-of-flight mass spectrometer capable of high-mass accuracy measurements of biomolecules. The QSTAR will have both electrospray and MALDI sources.

Access

Open

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In addition to the instrumentation mentioned above, the center is dedicated to keeping the lab equipped with state-of-the-art MS, sample preparation, and data analysis technology as it becomes available. Each of the instruments will be equipped with an automated LC system to allow high-throughput mass spectrometric characterization. The Mass Spectrometry Center will work in close collaboration with the Separation Technology group headed by Dr. Haleem Issaq to develop the most effective LC/MS experiments necessary for each research project.

Separation Technology Group
Analytical Chemistry Laboratory (ACL)

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Methods for sample clean-up and reagent extraction

Mission: The Separation Technology Group's mission is the development of high-resolution separation (GC, HPLC and CE) and ultra-sensitive detection methods that are reproducible, quantitative and efficient to meet the needs of NCI researchers.

Training courses in GC, HPLC and CE are offered to the users.

Expertise: The Separation Technology Group (STG) is staffed with experts in methods development for sample clean-up, extraction, separation, detection and quantitation of small molecules as well as large bio-molecules from biological, environmental and other matrices, with state-of-the-art instrumentation and techniques. We have been instrumental in developing methods for separation and quantitation, using GC, HPLC, CE and multidimensional methods of various compounds from complex biological mixtures. Our group developed UV laser-induced fluorescence for the sensitive detection of native proteins, peptides and amino acids, and capillary electrophoresis and GC columns to meet the needs of the researchers at NCI.

Training of users in different aspects and methods of separation is also part of our mission.

Established Technologies: The STG has been involved in the determination of DNA mutants by DHPLC; determination of deuterated glucose and deoxyadenosine from cell extracts by HPLC and GC/MS; separation of caffeine, nicotine and dextromethorphan metabolites in human urine by HPLC; separation of polychlorinated biphenyl congeners from human breast tissue by GC; purity determination of proteins, peptides, monoclonal antibodies by CE; determination of nucleocapsid protein/drug interaction and protein/DNA interaction by CE; fast separation of DNA fragments and PCR products using short CE capillaries; development of two-dimensional GC/GC for the separation of PCBs, and HPLC/CE for the separation of complex biological mixtures such as protein digests; development of ultra-sensitive laser fluorescence detection for DNA, proteins, peptides, amino acids, and the construction of novel columns for GC, HPLC and CE separations; quantitation of nitrosamines and nitrosoamino acids and other compounds of interest to cancer researchers from urine and gastric juice by GC.

New Technologies: Protein chip time-of-flight mass spectrometry with surface enhanced desorption and ionization-MS has been under

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evaluation since February 2001. Approximately 25 projects have been submitted, results on some of which are encouraging. This service is now available to the researchers.

A multidimensional separation technique using HPLC off-line with capillary electrophoresis for the separation of cell and tissue proteins has been developed.

Collaborations:

Dr. Michael Dean, LGD, NCI-Frederick.

Dr. Robert Fisher, PCL, SAIC, NCI-Frederick.

Dr. Howard Young, LEI, NCI-Frederick.

Publications:

Journal Articles:

Issaq HJ. The role of separation science in proteomics research. Electrophoresis 22:3629-3633, 2001.

Chan KC, Muschik GM, Issaq HJ. Solid-state UV laser-induced fluorescence detection in capillary electrophoresis. Electrophoresis 21:2062-2066, 2000.

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Yuan H, Janini GM, Issaq HJ, Thompson RA, Ellison DK. Separation of closely related heptapeptides by micellar electrokinetic chromatography. J. Liq. Chromatogr. 23:127-143, 2000.

Janini GM, Saptharishi N, Waselus M, Soman G. Element of a validation method for MU-B3 monoclonal antibody using an imaging capillary isoelectric focusing system. Electrophoresis, submitted 2001.

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Issaq HJ, Chan KC, Liu C, Li Q. Multidimensional high performance liquid chromatography/capillary electrophoresis separation of a protein digest: An update. Electrophoresis 22:1133-1135, 2001.

Janini GM, Metral CJ, Issaq HJ. Peptide Mapping by C2E: How close is theoretical simulation to experimental determination? J. Chromatogr. A. 924:291-306, 2001.

Chapters in Books:

Janini GM, Issaq HJ. Selection of buffers in capillary zone electrophoresis: Application to peptide and protein analysis. In: *CE in Biotechnology: Practical Applications for Protein and Peptide Analysis.* AB Chen, W Nashabeh, T Wehr, eds. Wiesbaden, Germany: H. Weinheimer, S18-S26, 2001.

Issaq HJ. Parameters affecting capillary electrophoretic separation of DNA. In: *Capillary Electrophoresis of Nucleic Acids*. Totawa, NJ: Humana Press, 189-199, 2000.

Issaq HJ, Chan KC. Fast DNA fragment sizing in a short capillary column. In: *Capillary Electrophoresis of Nucleic Acids*. Totawa, NJ: Humana Press, 41-45, 2000.

Issaq HJ. Gradient elution in capillary electrophoresis, micellar electrokinetic chromatography, capillary electrochromatography and microfluidics. In: *A Century of Separation Science*. HJ Issaq, ed. New York: Marcel Dekker, in press 2001.

Issaq HJ. A quarter century of separation science, from paper chromatography to capillary electrophoresis. In: *A Century of Separation Science*. HJ Issaq, ed. New York: Marcel Dekker, in press 2001.

Issaq HJ. Multimodal chromatography and gel electrophoresis. In: *A Century of Separation Science*. HJ Issaq, ed. New York: Marcel Dekker, in press 2001.

Issaq HJ. Solvent selection for optimal separation in liquid chromatography. In: *A Century of Separation Science*. HJ Issaq, ed. New York: Marcel Dekker, in press 2001.

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Janini GM. Liquid crystals stationary phases in gas chromatography: A historic prospective. In: *A Century of Separation Science*. HJ Issaq, ed. New York: Marcel Dekker, in press 2001.

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Nuclear Magnetic Resonance Group

Analytical Chemistry Laboratory (ACL)

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NMR data acquisition and analysis; NMR and ESR hardware design and construction

Mission: The ACL Nuclear Magnetic Resonance Group provides the NCI and its collaborators with cutting-edge NMR experiments and analysis using recently upgraded hardware and software. The expertise of this group offers flexibility in electronics engineering, pulse sequence programming, database access, including training and NMR spectral analysis at all levels. It is our goal to serve the NCI scientific community from simple data acquisition to taking a structural problem from the scientist's bench to publication. NMR training courses are offered to users.

Expertise: NMR (Nuclear Magnetic Resonance), a powerful tool for the determination of 3D structures in solution, has benefitted from the explosion of technology in the fields of electronics, computer science and quantum mechanics.

The ACL NMR Group in Research Technologies Program has recently undergone major upgrades and improvements in capabilities, including:

- High resolution magic angle spinning probe, nanoprobe
- Four-channel 500 MHz INOVA console and probes
- New 400 MHz spectrometer and probes
- ACD carbon database and predictor
- 500 MHz cryogenic probe on order.

In addition, the NMR Group maintains the same structure determination paradigm that uses indirect detection 1D and 2D methods and 1D versions of many of the 2D experiments; all of the software packages we have always offered; and a new and improved version of the in-house analysis package.

We invite you to take advantage of the expertise of our PhD engineer who has designed many NMR and ESR electronic improvemements. Our PhD pulse sequence programmer is available to design and implement new pulse sequences.

Established Technologies: To see examples of these experiments, go to the following website:

http://web.ncifcrf.gov/rtp/labs/ACL/nmr/exper.asp

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Routine 1D Spectra:

^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P

Through-Bond Correlation Experiments: Connectivity

COSY (COrrelation SpectroscopY)
TOCSY (TOtal Correlation SpectroscopY)
HSQC (Heteronuclear Single Quantum Correlation)
HMQC (Heteronuclear Multiple Quantum Correlation)
HMBC (Heteronuclear Multiple Bond Correlation)

Through-Space Correlation: Distance Information

TrROESY (Transverse Rotating Frame Overhauser Enhancement SpectroscopY)

NOESY (Nuclear Overhauser Enhancement SpectroscopY)

New Technologies Under Development: SOFTWARE Available:

PERCH – Spin simulation
ACD – ^{13}C database, ^{13}C predictor
ZFD – In-house program for data calculation and table assembly
NMRSAMS – Structure generation of small molecules from NMR data
NUTS – PC workup program, 1D version only
CHEMDRAW ULTRA – Draws structures, predicts molecular weight, and predicts chemical shifts

New Pulse Sequences Under Development:

Contact Dr. Que Van for current list.

NANO probe – has been installed and tested with small samples, beads andand viscous samples. Animal tissues will be tested. This probe is an H/X probe with magic angle spinning; is ideal for obtaining spectra of compounds attached to resin heads, and has a sample size limited to 40 uL.

CRYO Probe – has been ordered and should be delivered by November 2002 or sooner.

Collaborations:

Dr. Ruoli A. Bai, CCR, NCI. Proteins 1D, 2D and 3D.

Dr. Larry Keefer, LCC, NCI-Frederick. Kinetics and analysis.

Dr. Victor Marquez, LMC, NCI-Frederick. 1D, 2D including nOe and analysis.

Mazzola, UMD, FDA. Applications of NMR for textbook.

Dr. Thomas McCloud, SAIC, DPT, NCI-Frederick. Identification of unknown natural products.

Dr. Chris Michejda, DRP, NCI-Frederick. Unknown metabolites.

Publications:

Koscielniak J, Moni MS, Devasahaya N, Kuppusamy P, Yamada K, Mitchell JB, Krisha MC, Subramanian S. 300 MHz CW EPR spectrometer for small animal in vivo imaging. Review of Scientific Instruments, 71(11):4273-4281, 2000.

*Bohle DS, Bonifant CL, Chmurny GN, Flippen-Anderson JL, Keefer LK, Klose JR, *Saavedra JE. manuscript in preparation.

Nuclear Magnetic Resonance MiniCore

Laboratory of Medicinal Chemistry (LMC)

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NMR conformational analysis of small molecules

Mission: Research projects performed by the facility have primarily focused on the conformational analysis of small-molecule analogues of nucleosides, carbohydrates and peptides and the relationship of their structures with biological activity. This research direction complements the overall mission of the LMCH to pursue structure-based design of small molecules as potential antitumor and antiviral agents. The ultimate goal of the NMR MiniCore is to provide structural clues to the mechanisms of antitumor or antiviral activity of therapeutic candidates generated in the LMC or elsewhere within the NCI to guide the discovery and refinement of new therapies against cancer and HIV infection.

Expertise: The NMR MiniCore houses two high-field instruments at 400 and 500 MHz, respectively. The 400 MHz instrument is maintained as a walkup for all NCI researchers. The facility provides support in NMR experimental setup, protocol and interpretation. The high-field instrument is used for challenging structural problems generated either in the LMC, through collaborations with intra-and extramural researchers, or proposed by the facility head as original research. The 400 MHz instrument is a general use machine where users are trained to operate the instrument in an automated fashion. The facility head and staff are responsible for:

Training of new users in instrument operations and facility protocol

Experiment setup and implementation

Consultation and expert opinion on complex NMR problems

NMR hardware and software support.

Established Technologies: All areas of NMR spectroscopy (all experiments use pulse-field gradient technology), including:

Standard and selective 1D experiments, inverse detection

^{1}H, ^{13}C, ^{19}F and ^{31}P detection on the 400 MHz instrument; ^{1}H, ^{13}C, ^{15}N and broadband, inverse detection, ^{19}F observe and decouple on the 500 MHz instrument

All 2-D experiments, including COSY, NOESY, ROESY, TOCSY, HMQC, HMBC, HSQC, etc.

Decoupling experiments

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Post-doctoral Fellow (recruiting)

Technician (recruiting)

3D experiments used for protein structure determination

Heteronuclear NOEs (HOESY)

New Technologies Under Development:

Proton detected heteronuclear ^{1}H-^{19}F NOE experiments with WATERGATE H_2O suppression

H-F dipolar coupling determination using spin state selective E-COSY type experiments

TROSY-type experiments for larger protein-ligand complexes

Collaborations:

Dr. Robert Blumenthal, NCI-Frederick. Experimental and Computational Biology.

Dr. David Roberts, NCI. Pathology.

Sam Gellman, University of Wisconsin.

Bert Fraser-Reid, NPG, Durham, NC.

Greg Helms (Washington State U., Pullman, WA).

Publications:

Mu L, Sarafianos SG, Nicklaus MC, Russ P, Siddiqui MA, Ford H Jr, Mitsuya H, Le R, Kodama E, Meie C, Knipsel T, Anderson L, Barchi JJ Jr, Marquez VE. Interactions of conformationally-biased north and south 2´-fluoro-2´,3´dideoxynucleoside 5´-triphosphates with the active site of HIV-1 reverse transcriptase. Biochemistry 39:11205-11215, 2000.

Ford H Jr, Dai F, Mu L, Siddiqui MA, Nicklaus MC, Anderson L, Marquez VE, Barchi JJ Jr. Adenosine deaminase prefers a distinct sugar conformation for binding and catalysis: kinetic and structural studies. Biochemistry. 39:2581-2592, 2000.

Siddiqui MA, Driscoll JS, Abushanab E, Kelley JA, Barchi JJ Jr, Marquez VE. The "b-Fluorine Effect" in the non-metal hydride radical deoxygenation of fluorine-containing nucleoside xanthates. Nucleos. Nucleot. Nucleic Acids 19:1-12, 2000.

Yao Z-J, Barchi JJ Jr, Burke TR Jr. Design and synthesis of a new tyrosine analogue having c1 and c2 angles constrained to values observed for an SH2 domain-bound phosphotyrosyl residue. In: *Peptides for the New Millennium*. (Proc 16th Amer. Peptide Symposium). Fields GB, Tam JP, Barany G. (Eds) Dordrecht, The Netherlands: Kluwer Acad. Publishers, 575-578, 2000.

Barchi JJ Jr, Huang X, Appella DH, Christianson LA, Durell SR, Gellman SH. Solution conformations of helix-forming β-amino acid homo-oligomers. J. Am. Chem. Soc. 122:2711-2718, 2000.

Luy B, Marino JP. Measurement and application of ^{1}H-^{19}F dipolar couplings in the structure of 2´-fluoro-labeled RNA. J. Biomol. NMR. 20:39-47, 2001.

Luy B, Barchi JJ Jr, Marino JP. S(3)E-E.COSY methods for the measurement of ^{19}F associated scalar and dipolar coupling constants. J. Magn. Res. 152, in press 2001.

Animal Health Diagnostic Laboratory

Laboratory Animal Sciences Program (AHDL/LASP)

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Monitoring rabbit and rodent health

Mission: The mission of the Animal Health Diagnostic Laboratory (AHDL) is to monitor the health of rodents and rabbits used in biomedical research at the NCI and other government agencies. Our goal is to determine which animals may be infected with various pathogens and could pose a threat to other animals in the facilities. In collaboration with LASP and LAM offices, we recommend a course of action to prevent or mitigate an outbreak and preserve and protect animal health and biomedical research.

Expertise: The AHDL is responsible for the health monitoring of all rodents and rabbits at NCI-Frederick, NCI-Bethesda, NCI-DTP Rodent Producers and several NCI contract facilities. The lab also provides diagnostic services to several other NIH animal facilities and to the U.S. Army Medical Research Institute of Infectious Diseases. The major focus of diagnostic services includes bacteriology, parasitology, serology, molecular detection of pathogens and necropsies. The lab works closely with Animal Holding and Technical Support (AHTS), Laboratory Animal Medicine (LAM), and the Pathology/Histotechnology Laboratory (PHL) to provide a comprehensive approach to health monitoring. The AHDL has over 25 years of experience in the research community and has been in the forefront of rodent diagnostics, including the detection and original isolation of the recently discovered *Helicobacter hepaticus*, a significant rodent pathogen.

Established Technologies:

Serology

Bacteriology

Parasitology

Molecular Diagnostics/PCR

Necropsy

New Technologies Under Development: Replacement of the in vivo MAP/RAP test with a molecular-based assay

Access

Lab/Program Dedicated

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Cryopreservation and Assisted Reproduction Program

Cryo Lab

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State-of-the-art technologies for cryopreservation

Mission: The LASP/Cryo program provides NCI investigators a new option to manage those mouse colonies that are not used in research anymore but are important enough to keep alive. By freezing them, they are protected from genetic drift, spontaneous mutations, contaminations and disease outbreaks.

Expertise: The Cryopreservation Program provides state-of-the-art technologies for cryopreserved mouse strains by embryo, sperm or ovary freezing.

The Assisted Reproduction Techniques (ART), in-vitro fertilization (IVF), ovary maturation (OM), are applied to colony expansion and rederivation to overcome infertility problems and rescue valued animals.

Established Technologies: The Cryopreservation Program offers the following cryopreservation techniques:

Embryo: Slow freezing

Ovary: Slow freezing

Sperm freezing

Vitrification

In-vitro fertilization (IVF), colony expansion, rederivation

Embryo transfer

Ovary transfer

Ovary maturation

Sperm selection

Sperm analysis

New Technologies Under Development:

Intra-cytoplasmatic sperm injection (ICSI)

Nuclear transfer

Collaborations:

Dr. Gabor Vajda, Monarsch University, Australia. Ongoing mouse oocyte vitrification study.

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Publications:

Sztein JM, O'Brien MJ, Farley JS, Mobraaten LE, Eppig JJ. Rescue of oocytes from antral follicles of cryopreserved mouse ovaries: competence to undergo maturation, embryogenesis, and development to term. Hum. Reprod. 15(3):567-571, 2000.

Sztein JM, Farley JS, Mobraaten LE. In vitro fertilization with cryopreserved inbred mouse sperm. Biol. Reprod. 63:1774-1780, 2000.

Sztein JM, Noble K, Farley JS, Mobraaten LE. Comparison of permeating and nonpermeating cryoprotectants for mouse sperm cryopreservation. Cryobiology. 41:1-12, 2001.

Animal Holding and Technical Support
Laboratory Animal Sciences Program (AHTS/LASP)

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Animal care and animal support services for NCI

Mission: The mission of the Laboratory Animal Sciences Program (LASP) is to provide the highest quality animal care and animal support services for all animal research at NCI-Frederick and NCI-Bethesda. The function of the Animal Holding and Technical Support program is twofold: (1) to ensure that the investigators' needs are met through the use of healthy animals appropriate for the research requirement and that all animals are housed, handled, and cared for in a humane manner in a controlled environment; and (2) to provide scientific support for researchers performing animal-based research. The LASP, with a staff of 185, provides a central point to meet, address, and support the animal requirements of both government and contractor in biomedical research activities at NCI-Frederick and NCI-Bethesda. The LASP works closely with the NCI Office of Laboratory Animal Science and the NCI-Frederick Animal Care and Use Committee (IACUC) in developing, implementing, and monitoring the programs and facilities of NCI-Frederick for activities involving animals.

Expertise:

- Animal care
- Veterinary medicine
- Surgical procedures
- Specialized animal handling procedures
- Chemical carcinogenesis
- Transgenic mouse production
- Breeding to establish congenic strains
- Irradiation
- Immunology
- Tumor transplantation
- Antibody production for activities involving animals
- Developmental biology

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DNA vaccines (Gene Gun)

SIV, HIV, HTLV, antigen capture assays

Hollow fiber assays (drug testing)

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New Technologies Under Development: Animal holding and research rules and regulations are always being updated; we continue to keep up with these changes. Requests from the investigators are always changing and we continue to keep abreast of procedures and equipment to be able to provide for the principal investigators' needs.

Receiving and Quarantine Facility
(R&Q/LASP)

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Test and quarantine of animals to be housed at NCI-Frederick

Mission: The Receiving and Quarantine (R&Q) mission is to provide investigators at the NCI-Frederick with animals of acceptable health status through the quarantine and testing of animals to detect and exclude animals that may be harboring undesirable organisms. Along with this service, R&Q can import animals with undesirable pathogens and rederive them to SPF status, thus allowing investigators access to virtually any strain that would historically be excluded from the NCI-Frederick facility.

Expertise: R&Q receives animals from various vendors and institutions and quarantines them for various lengths of time based on the origin of the shipment.

Located within the R&Q facility is an area for housing mice with known disease organisms. Animals are held in this area for a finite amount of time until they can be rederived and clean animals can be released into an NCI-Frederick animal colony. The R&Q Rederivation Program has been in operation since 1999, and is available to any investigator or collaborator, with a current approved ASP, conducting research at NCI-Frederick. As part of the Rederivation Program, the R&Q facility imports animals in cooperation with the MMHCC for rederivation. After rederivation and testing, animals for the MMHCC are then released to the APA for expansion and distribution to investigators.

Established Technologies:

Quarantine of mice and rats, daily husbandry, and collection of samples for endoparasitology, ectoparasitology, bacteriology, and serology

Mating, weaning and identification (ear-tag, or notch) of animals in quarantine

Rederivation of mice using Embryo Transfer

Collection of samples for DNA analysis (tail clips, ear punch)

Collection of tissues at investigator request

New Technologies Under Development:

In-vitro fertilization for rederivation

Acceptance of frozen gametes, embryos or reproductive tissues for rederivation.

Access

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Transgenic Mouse Model
(LASP)

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Research Technology Program and Laboratory Animal Sciences Program, SAIC-Frederick, Inc.

Web page: http://web.ncifcrf.gov/rtp/LASP.asp

Transgenic mouse production

Mission: The Transgenic Mouse Model (TMM) laboratory provides NCI investigators with a state-of-the-art facility to develop transgenic animals to study in vivo gene function. In addition to the technical expertise required for this task, the laboratory offers assistance with transgenic project development and characterization of transgenic mice.

Expertise: The Transgenic Mouse Model (TMM) laboratory produces transgenic mice by pronuclear microinjection of fertilized mouse eggs. Although this service is provided on an individual basis, the core of this effort includes purification of the fragment to be microinjected, microinjection and analysis of the resulting mice by Southern blot, PCR or slot/blot. The laboratory provides consultations in the design of the recombinant DNA constructs for use in transgenic mouse studies. Characterization of the expression pattern of the transgene at the RNA or protein level is also available.

Established Technologies:

Derivation of transgenic mice/pronuclear microinjection

Genotypic characterization of transgenic mice/Southern analysis, PCR analysis

Phenotypic characterization of transgenic mice, Northern analysis, Western analysis, RT-PCR analysis.

New Technologies Under Development:

Derivation of transgenic rats by pronuclear microinjection.

Collaborations:

The Transgenic Mouse Model (TMM) laboratory is involved in collaborations with the PIs listed below. These collaborations involve assistance with phenotypic characterization of mice generated by TMM and intellectual input in projects.

Dr. Alfred Singer, EIB, CCR, NCI.

Dr. Dolph Hatfield, Laboratory of Metabolism, CCR, NCI.

Dr. Nicholas Restifo, Surgery Branch, CCR, NCI.

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Dr. Maria Zajac-Kaye, Medicine Branch, Genetics Department, CCR, NCI.

Dr. Barbara Vonderhaar, Laboratory of Tumor Immunology and Biology, CCR, NCI.

Publications:

Bosselut R, Kubo S, Guinter T, Kopacz JL, Altman JD, Feigenbaum L, Singer A. Role of CD8b domains in CD8 coreceptor function: importance for MHC signaling, and positive selection of $CD8^+$ T cells in the thymus. Immunity. Apr; 12(4):409-418, 2000.

Marks-Konczalik J, Dubois S, Losi JM, Sabzevari H, Yamada N, Feigenbaum L, Waldmann TA, Tagaya Y. IL-2-induced activation-induced cell death is inhibited in IL-15 transgenic mice. Proc. Natl. Acad. Sci. USA. Oct 10; 97(21):11445-11450, 2000.

Kovalchuk AL, Qi CF, Torrey TA, Taddesse-Heath L, Feigenbaum L, Park SS, Gerbitz A, Klobeck G, Hoertnagel K, Polack A, Bornkamm GW, Janz S, Morse HC III. Burkitt lymphoma in the mouse. J. Exp. Med. Oct 16; 192(8): 1183-1190, 2000.

Bosselut R, Feigenbaum L, Sharrow SO, Singer A. Strength of signaling of Cd4 and Cd8 coreceptor tails determines the number but not the lineage direction of positively selected thymocytes. Immunity. Apr; (14):483-494, 2001.

Moustafa M, Carlson B, el-Saadani M, Kryukov G, Sun Q, Harney J, Hill K, Combs G, Feigenbaum L, Mansur D, Burk R, Berry M, Diamond A, Lee B, Gladyshev V, Hatfield D. Selective inhibition of selenoprotein sythesis in transgenic mice expressing isopentenyladenosine-deficient tRNA. Mol. Cell. Biol. Jun; 21(11):3840-3852, 2001.

Bodor J, Feigenbaum L, Bodorova J, Bare C, Reitz M, Gress R. Suppression of T-cell responsiveness by inducible cAMP early repressor (ICER). J. Leukoc. Biol. Jun; 69(6):1053-1056, 2001.

Conchero J, Grandvil C, Akiyama T, Hayhurst G, Pimprale S, Feigenbaum L, Idle J, Gonzalez F. The CYP2D6 humanized mouse: effect of the human CYP2D6 transgene and HNF4alpha on the disposition of debrisoquine in the mouse. Mol. Pharmacol. Dec; 60(6):1260-1267, 2001.

Transgenic Core Facility

Mouse Cancer Genetics Program (MCGP)

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Mouse Cancer Genetics Program, CCR, NCI-Frederick

Consultation and technical services in the design, construction, and breeding of transgenic mice

Mission: The Transgenic Core Facility creates mouse models of human diseases pertinent to research at NCI-Frederick. The facility interacts with scientific staff to discuss production methods of transgenic mice as well as the directions of research studies.

Expertise: The facility provides consultation and technical services in experimental design, construction, and breeding of transgenic mice through the use of microinjection techniques, to include plasmids, PACs, and BACs. The facility develops and modifies various techniques to suit the needs of the current research in the Transgenic Core Facility.

Other Services Provided:

Tissue collection, tail clips, ovary transfers, embryo transfers, vasectomies, splenectomies and timed pregnancies.

Established Technologies:

Produce transgenic mice using plasmids, BACs and PACs.

Produce specialty media, microinjection needles, holding pipettes, transfer pipettes.

New Technologies Under Development:

We are working on techniques to make the microinjection of plasmids, BACs and PACs more efficient.

Publications:

Moore KJ, Swing DA, Rinchik EM, Mucenski ML, Buchberg AM, Copeland NG, Jenkins NA. The murine dilute suppressor gene, *dsu*, suppresses the coat-color phenotype of three pigment mutations that alter melanocyte morphology, d, ash, and In. Genetics 119:933-941, 1988.

Moore KJ, Seperack PK, Strobel MC, Swing DA, Copeland NG, Jenkins NA. Dilute suppressor, dsu, acts semidominantly to suppress the coat color phenotype of a deletion mutation, dl20J, of the murine dilute locus. Proc. Natl. Acad. Sci. USA 85:8131-8135, 1988.

Copeland NG, Lock LF, Spence SE, Moore KJ, Swing DA, Gilbert DJ, Jenkins NA. Ecotropic murine leukemia viruses: Insertional mutagens and vectors for introducing foreign genes into the mammalian germline. Mouse News Lett. 82:100, 1988.

Access

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Spence SE, Gilbert DJ, Swing DA, Copeland NG, Jenkins NA. Spontaneous germline virus infection and retroviral insertional mutagenesis in eighteen transgenic Srev lines of mice. Mol. Cell. Biol. 9:177-184, 1989.

Copeland NG, Lock LF, Spence SE, Moore KJ, Swing DA, Gilbert DJ, Jenkins NA. Spontaneous germline ecotropic MuLV infection: Implications for retroviral insertional mutagenesis and germline gene transfer. In: *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 36, "Transposable Elements in Mutagenesis and Regulation of Gene Expression" (W Cohn, ed.). Orlando, FL: Academic Press, Inc., pp. 221-234, 1989.

Copeland NG, Buchberg AM, Ceci JD, Cho B, Cybulski MB, Gilbert DJ, Justice MJ, Kingsley DM, Lock LF, Mercer JA, Moore KJ, Perkins A, Silan CM, Siracusa LD, Spence SE, Strobel MC, Swing DA, Jenkins NA. Establishment and applications of a molecular genetic linkage map of the mouse genome. Mouse News Lett. 85:83, 1989.

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Moore KJ, Swing DA, Copeland NG, Jenkins NA. Interaction of the murine dilute suppressor gene (*dsu*) with fourteen coat color mutations. Genetics 125:421-430, 1990.

Ceci JD, Kovatch RM, Swing DA, Jones JM, Snow CM, Jenkins NA, Copeland NG, Rosenberg MR, Meisler MH. Transgenic mice carrying a murine amylase 2.2/SV40 T antigen fusion gene develop pancreatic acinar cell and stomach carcinomas. Oncogene 6:323-332, 1991.

Moore KJ, Swing DA, Copeland NG, Jenkins NA. The murine dilute suppressor gene encodes a cell autonomous suppressor. Genetics 138:491-497, 1994.

Siracusa LD, Washburn LL, Swing DA, Argeson AC, Jenkins NA, Copeland, NG. Hypervariable yellow (Ahvy), a new murine agouti mutation: Ahvy displays the largest variation in coat color phenotypes of all known agouti alleles. J. Hered. 86:121-128, 1995.

Perry WL, Hustad CM, Swing DA, Jenkins NA, Copeland NG. A transgenic mouse assay for agouti protein activity. Genetics 140:267-274, 1995.

Perry WL, Nakamura T, Swing DA, Secrest L, Eagleson B, Hustad CM, Copeland NG, Jenkins NA. Coupled site-directed mutagenesis/transgenesis identifies important functional domains of the mouse agouti protein. Genetics 144:255-264, 1996.

Federspiel MJ, Swing DA, Eagleson B, Reid SW, Hughes SH. Expression of transduced genes in mice generated by infecting blastocysts with avian leukosis virus-based retroviral vectors. Proc. Natl. Acad. Sci. USA 93:4931-4936, 1996.

Ceci JD, Patriotis CP, Tsatsanis C, Makris AM, Kovatch R, Swing DA, Jenkins NA, Tsichlis PN, Copeland NG. Tpl-2 is an oncogenic kinase that is activated by carboxy-terminal truncation. Genes Dev. 11:688-700, 1997.

Perry WL, Hustad CM, Swing DA, O'Sullivan N, Jenkins NA, Copeland, NG. The itchy locus encodes a novel ubiquitin protein ligase that is disrupted in a18H mice. Nat. Genet. 18:143-146, 1998.

Wilson SM, Yip R, Swing DA, O'Sullivan N, Zhang Y, Novak EK, Swank RT, Russell LB, Copeland NG, Jenkins NA. A mutation in Rab27a causes the vesicle transport defects observed in ashen mice. Proc. Natl. Acad. Sci. USA 97:7933-7938, 2000.

Lee E-C, Yu D, Martinez de Velasco J, Swing DA, Tessarollo L, Court DL, Jenkins NA, Copeland NG. A highly efficient *E. coli*-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. Genomics 73:56-65, 2001.

Chandler J, Hohenstein P, Swing DA, Tessarollo L, Sharan SK. Human *BRCA1* gene rescues the embryonic lethality of Brca1 mutant mice. Genesis 29:72-77, 2001.

Gene Targeting Facility

Mouse Cancer Genetics Program (MCGP)

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Mouse Cancer Genetics Program, CCR, NCI-Frederick

Targeting stem cells to provide mouse models of disease

Mission: The Gene Targeting Facility develops mouse models with specific targeted mutations.

Expertise: The Gene Targeting Facility provides support to the investigators to generate gene-targeted mutations in mice. One goal is to help the investigator choose the most suitable model according to his/her scientific need. Expertise can be provided in the following areas: 1) intellectual support in vector design; 2) embryonic stem cell manipulations; 3) blastocyst injections; 4) breeding of chimeras to germline transmission.

Established Technologies:

Handling of embryonic stem (ES) cells without affecting their totipotency

Manipulation of ES cells, including electroporation, selection of recombinant clones and cell expansion

Microinjection of blastocysts and production of chimeric mice

Generation of backbone vectors useful in making constructs for gene targeting.

New Technologies Under Development:

Generation of vectors to create conditional alleles.

Mouse lines expressing the cre recombinase in a general or tissue-specific manner are also being developed.

Publications:

Choi T, Fukasawa K, Zhou R, Tessarollo L, Borror K, Resau J, Vande Woude GF. The mos/mitogen-activated protein kinase (MAPK) pathway regulates the size and degradation of the first polar body in maturing mouse oocytes. Proc. Natl. Acad. Sci. 93:7032-7035, 1996.

Sterneck E, Tessarollo L, Johnson PF. An essential role for C/EBPβ in female reproduction. Genes Dev. 11:2153-2162, 1997.

Steingrímsson E, Tessarollo L, Reid SW, Jenkins NA, Copeland NG. The bHLH-Zip transcription factor Tfeb is essential for placental vascularization. Development 125:4607-4616, 1998.

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Sterneck E, Paylor R, Jackson-Lewis V, Libbey M, Przedborski S, Tessarollo L, Crawley JN, Johnson PF. Selectively enhanced contextual fear conditioning in mice lacking the transcriptional regulator CCAAT/Enhancer binding protein. Proc. Natl. Acad. Sci. 95:10908-10913, 1998.

Taylor GA, Collazo CM, Yap GS, Nguyen K, Gregorio TA, Taylor LS, Eagleson B, Secrest L, Southon EA, Reid SW, Tessarollo L, Bray M, McVicar DW, Komschlies KL, Young HA, Biron CA, Sher A, Vande Woude GF. Pathogen-specific loss of host resistance in mice lacking the IFN-γ-inducible gene IGTP. Proc. Natl. Acad. Sci. (USA). 97:751-755, 2000.

Esteban LM, Fernández-Medarde A, López E, Yienger K, Guerrero C, Ward JM, Tessarollo L, Santos E. Ras-guanine nucleotide exchange factor Sos2 is dispensable for mouse growth and development. Mol. Cell. Biol. 20:6410-6413, 2000.

Wilson SM, Toth PT, Oh SB, Gillard SE, Volsen S, Ren D, Philipson LH, Lee EC, Fletcher CF, Tessarollo L, Copeland NG, Jenkins NA, Miller RJ. The status of voltage-dependent calcium channels in α 1E knock-out mice. J. Neurosci. 20:8566-8571, 2000.

Bonin A, Reid SW, Tessarollo L. Gene knockouts: Isolation microinjection and transfer of mouse blastocysts. Methods Mol. Biol. 158:121-134, 2001.

Tessarollo L. Gene knockouts: Manipulating mouse embryonic stem cells. Methods Mol. Biol. 158:47-63, 2001.

Zhu J, Petersen S, Tessarollo L, Nussenzweig A. Targeted disruption of the Nijmegen Breakage Syndrome gene *NBS1* leads to early embryonic lethality in mice. Cur. Biol. 11:105-109, 2001.

Fletcher CF, Tottene A, Lennon VA, Wilson SM, Dubel SJ, Paylor R, Hosford DA, Tessarollo L, McEnery MW, Pietrobon D, Copeland NG, Jenkins NA. Dystonia and cerebellar atrophy in Cacna1a null mice lacking P/Q calcium channel activity. FASEB J. 15:1288-1290, 2001.

Geiman TM, Tessarollo L, Anver MR, Kopp JB, Ward JM, Muegge K. Lsh, a SNF2 family member, is required for normal murine development. Biochim. Biophys. Acta. 1526:211-220, 2001.

Germline Mutation Core Facility
GMCF

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Production of genetically altered mice and development of novel techniques

Mission: The Germline Mutation Core Facility (GMCF) provides the CCR/NCI community with services for producing genetically altered mice.

Expertise: Our function as a core facility is focused in the following areas:

Consultation on the design of gene-targeting vectors

Production of transgenic animals by

Injecting DNA fragment into zygotes

Introducing the DNA into embryonic stem (ES) cells, selecting for cells in which the DNA has undergone homologous recombination with matching genomic sequences, and injecting positive ES clones into blastocysts.

Development of new techniques for making genetically engineered animals.

The Germline Mutation Core Facility (GMCF) was established in 1999 in response to the need for such transgenic technologies by NCI investigators.

Established Technologies:

Embryonic stem (ES) cell culture, electroporation of targeting vector, selection of drug-resistant ES clones

Microinjection of ES cells into mouse blastocysts

Microinjection of DNA into mouse zygotes.

New Technologies Under Development:

GMCF is open to new ideas and will try to support and develop novel, but not well-established procedures. Since such projects are unique and much more time-consuming, they will require collaboration between researchers and our facility.

Procedures Currently Under Development:

Production of C57BL/6 chimera with C57BL/6 ES cells into wild-type or albino C57BL/6 strains

Production of completely ES-derived fetuses by aggregation with tetraploid embryos

Microinjection of double-stranded RNA (RNAi) into mouse zygotes.

Access

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Collaborations:

Dr. Albert Fornace, BRL, NCI. Rescue of p38α lethal knockout by tetraploid embryos technique.

Dr. Liya Shen, LCCTP, CCR, NCI. Involvement of novel GDNF/c-Ret downstream molecules during kidney organogenesis using siRNA.

Mouse Models of Human Cancer Consortium Repository

Laboratory Animal Sciences Program (MMHCC/LASP)

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Laboratory Animal Sciences Program, SAIC-Frederick, Inc.

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Repository for mouse cancer models of human cancers and associated strains

Mission: The repository makes important genetically engineered mouse models of human cancer strains, and associated strains, available to NCI MMHCC members, NCI investigators, and all other members of the scientific community.

Expertise: The Mouse Models of Human Cancer Consortium (MMHCC) Repository is an NCI-funded repository for mouse cancer models of human cancers and associated strains. Strains are selected for the repository by MMHCC Repository committee members. All strains are rederived prior to distribution to insure the highest health. Embryos or gametes are cryopreserved to allow efficient management of stocks and protect against accidental loss. The genetic quality of all strains is monitored with respect to the specific mutation carried by a strain as well as the strain genetic background. Technical information of all repository is maintained and is available from the repository web site.

Strains are kept as live animals for a period of 6 months, after which time their distribution level is evaluated. Those strains that receive little or no interest are removed from the shelf, but are available for recovery from frozen stocks.

Established Technologies:

- Mouse genetics
- Mouse husbandry practices, particularly with relevance to genetically engineered mice
- Generation of congenic strains, including speed congenics
- Genotyping
- Genetic quality control for rodent strains
- Rederivation of mouse/rat strains
- Cryopreservation of mouse gametes, embryos and reproductive organs
- Recovery of cryopreserved strains

New Technologies Under Development:

Generation of speed congenics – marker-assisted backcrossing for generating congenic strains in a year or less.

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Molecular genetic quality control for mouse strains – protocols are being developed that will provide array-based technology for genome scans. These will be useful for both genetic background verification and speed congenic methodology.

The use of assisted reproduction to maintain and distribute mouse strain protocols to ship and recover frozen gametes or embryos is being developed.

Collaborations:

Dr. Jorge Sztein, SAIC, LASP, NCI-Frederick. Developing protocols to transport and recover frozen sperm.

Dr. David Monroe, SAIC, RTP, NCI-Frederick. Developing arrays for genome scans of mouse strains.

Publications:

Sharp JJ, Linder CC, Mobraaten LE. Genetically engineered mice, husbandry and resources. In: *Methods in Molecular Biology*, vol. 158. Tyms MJ, Kola I. eds. Totawa, NJ: Humana Press, Inc.

Sharp JJ, Sargent EE, Schweitzer PA. Genetic monitoring. In: *Laboratory Animal Medicine*, 2nd Edition. Fox J, ed. (in press).

Pathology/Histotechnology Laboratory
Laboratory Animal Sciences Program (PHL/LASP)

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Necropsy protocols and evaluation of tissues from research animals

Mission: The Pathology/Histotechnology Laboratory (PHL) at NCI-Frederick offers a full range of services including animal necropsies, routine and special histological preparations, histopathologic evaluation, and assistance in experimental design. The purpose of this diversified support group is to provide quality, flexible, and dependable histopathology service to NCI and NIH investigators requiring any of these procedures. Collaboration with scientific staff is highly encouraged, whether it is on the technical level regarding methods, or on a scientific level, requiring consultation with a staff pathologist. PHL is part of the Laboratory Animal Sciences Program under the direction of Dr. Hendrick Bedigian.

Expertise: Pathology services are offered by board-certified veterinary pathologists in the Veterinary Pathology Section of PHL. These services run the spectrum from design of necropsy protocols to pathology evaluation of tissues (slides) from experimental animals.

Preparation of individual animal reports and comprehensive study summary data

Quantitative pathology

Molecular pathology techniques

Photomicrography/telepathology.

The Histotechnology Laboratory provides an extensive range of histology services. In addition to slide preparation, the laboratory provides the following services:

Necropsy – detailed dissection and description of gross lesions

Blood, body fluids and tissue sampling

Cryotomy
– Special stains

Beta-Gal staining

Molecular histology techniques

Immunohistochemistry

In-situ hybridization

Access

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Tissue microarrays

Hematology – full hematology profile.

Established Technologies:

VETERINARY PATHOLOGY SECTION

Comprehensive pathology services are provided by PHL's board-certified veterinary pathologists. These services range from design of necropsy protocols, necropsy supervision and development of specialized necropsy and trimming procedures to pathology evaluation of tissues (slides) from experimental animal subjects with special emphasis on genetically engineered mice (GEM).

Automated pathology data base – Pathology data can be entered directly by the pathologist into the LABCAT® computerized pathology data base; this allows investigators to receive not only individual animal reports but also summary incidence tables of neoplastic and nonneoplastic lesions.

MOLECULAR PATHOLOGY

Immunohistochemistry – Pathologists establish conditions for optimizing new antibodies, double immunostaining techniques and provide interpretation of findings, including quantitation, e.g., microvessels, cell proliferation indices.

Apoptosis evaluation and quantitation.

Interpretation of histochemical and special stains.

PHOTOMICROGRAPHY/TELEPATHOLOGY

Pathologists provide investigators with photomicrography services using film and digital media. The latter can be transmitted via the Internet or stored on diskette or CD.

HISTOTECHNOLOGY LABORATORY

In slide preparation, the Histotechnology laboratory makes every effort to ensure excellent quality microslides with a prompt turnaround period. PHL prepares routine H&E sections as well as serial sectioning for in-situ hybridization and PCR analysis.

Services:

Necropsy

Blood collection

Cryotomy

Routine histology

Molecular histology

Necropsy:

Animal necropsies, complete with precise gross descriptions, are routinely performed by experienced prosectors under the direction of staff pathologists. Necropsy protocols are customized for each investigator's study.

Special tissue trimming protocols can be developed and executed according to the unique needs of any investigator and can be tailored to meet specific research requirements.

Fixation – PHL recommends the use of buffered formalin whenever possible; other fixatives for specialized procedures include:

Bouins fixative

Paraformaldehyde

Alcoholic fixatives

Zinc Formalin

Carnoy's

Tissue may also be collected in liquid nitrogen or frozen in OCT for cryotomy. Other techniques include:

Blood smears

Organ touch preparations

Blood serum collection

Bone marrow aspirates

Organ weights

Gross photography

Special Capabilities:

In addition to slide preparation with hematoxylin and eosin (H&E) staining, PHL also offers the following special procedures. (Some advance notice may be required for these services.)

Cryostat sectioning – High quality frozen sections from fresh or fixed materials; single, step serial, or serial sections.

Immunohistochemistry – Immunoperoxidase or alkaline phosphatase staining is performed using a variety of chromagens. Double stains are also performed. Immunostaining, using primary antibodies supplied by the investigator, can be performed on paraffin or frozen sections. Microwave and pressure cooker capability is available. The use of special reagent kits (ARK, Animal Research Kit, Dako and MOM, Mouse on Mouse, Vector) allows immunostaining of mouse tissues by mouse monoclonal antibodies without interfering background staining. An automated immunostainer is available to handle high volume with fast turn-around.

Apoptosis – Can be evaluated in paraffin or frozen sections using the Intergen Apoptag kits.

Histochemical procedures – Can be performed on cytospins, blood smears, paraffin or frozen sections. Examples of histochemical procedures offered are:

Chloracetate esterase

Nonspecific esterase

Acid and alkaline phosphatase

Tartrate-resistant acid phosphatase in bone in soft paraffin

Beta glutamyl transpeptidase

Lambda glucuronidase

Glucose-6-phosphatase

Special stains – Special staining techniques are available. A few of the commonly requested stains are listed here:

Stain	Demonstrates
Alcian Blue	Acid mucins
Beilschowsky	Nerve fibers
Congo Red	Amyloid
GMS	Fungi, *Pneumocystis*
Gram (Brown & Hopps)	Gram-positive & gram negative bacteria
Oil Red O	Fat (Cryostat sections)
PAS	Neutral mucins, Basement membranes
Sevier Munger	Argentaffin cells/ Nerve cells and fibers
Steiner (modified)	*Helicobacter*
Trichrome	Connective tissues
Toluidine Blue	Mast cells, cartilage
van Giesen	Connective tissues
von Kossa	Calcium/mineralized materials
Zeihl Neelsen	Acid fast bacteria

New Technologies Under Development: PHL is currently developing protocols for the following techniques:

Tissue Microarrays – "normal" mouse tissues in a range of fixatives for use in immunohistochemistry screening.

Glycolmethacrylate (GMA) plastic embedding, for enhanced morphology in a number of different applications.

Veterinary Pathology Section Collaborations:

PHL pathologists are prepared to function as scientific collaborators in keeping with NCI's research mission. They review literature relevant to the hypotheses being tested, assist with design of necropsy protocols, including selection of target organs and fixatives, and provide interpretation of pathology data, as well as tissue diagnoses. Interaction is facilitated by our on-site location for NCI-Frederick investigators and the shuttle service between NIH, Bethesda and Frederick, as well as phone, fax and electronic mail. PHL's staff is proactive in development of new techniques, including molecular pathology techniques (immunohistochemistry, apoptosis evaluation) to support research projects. Pathologists are available to interact with investigators at any phase of a study, including slide review at a dual-viewing microscope. Our contributions to various research projects are reflected by inclusion of PHL pathologists as co-authors on scientific manuscripts.

Examples of ongoing collaborations with NCI investigators are Dr. Anver's work with Dr. Glenn Merlino's HGF/SF transgenic mice as animal models

of malignant melanoma and rhabdomyosarcoma; Dr. Haines' long-term collaboration with Drs. Steven Hursting and Susan Perkins on cancer chemoprevention in genetically engineered mice; and the participation of Drs. Anver and Haines, Keith Rogers and the Molecular Histology area of PHL in activities of NCI's Mouse Mammary Models Collective, Dr. Jeffrey Green, Principal Investigator.

Publications:

Hixson JA, Blazar BR, Anver MR, Wiltrout RH, Murphy WJ. Antibodies to CD40 induce cytokine cascade after syngeneic bone marrow transplantation. ASBMT, in press 2001.

Geima TM, Tessarollo L, Anver MR, Kopp JB, Ward JM, Muegge K. Lsh, a SNF2 family member, is required for normal murine development and kidney function. Biochim. Biophy. Acta, General Subjects in press 2001.

Welniak LA, Khaled AR, Anver MR, Komschlies KL, Wiltrout RH, Durum S, Ruscetti FR, Blaar BR, Murphy WJ. Gastrointestinal cells of IL-7 receptor null mice exhibit increased sensitivity to irradiation. J. Immunol. 166:2923-2928, 2001.

Ward JM, Yoon M, Anver MR, Haines DC, Kudo G, Gonzalez FJ, Kimura S. Hyalinosis and Ym1/Ym2 gene expression in the stomach and respiratory tract of 129S4/SvJae and wild-type and CYP1A2-null B6, 129 mice. Am. J. Pathol. 158(1), 2001.

Josyula S, Schutt HAJ, Diwan BA, Anver MR, Anderson LM. Age-related alterations in ^{32}P-postlabeled DNA adducts in livers of mice infected with the tumorigenic bacterial pathogen, *Helicobacter hepaticus*. Int. J. Oncol. 17:811-818, 2000.

FACS Core Laboratory

Laboratory of Cellular Carcinogenesis and Tumor Promotion (LCCTP)

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FACS Core, CCR, NCI-Bethesda

Multiparameter flow cytometry and fluorescence-activated cell sorting

Mission: The FACS Core Lab provides state-of-the-art multiparameter flow cytometry and cell sorting facilities for studying cellular proteins, nucleic acids, and functional characteristics of live and fixed cells. The facility will perform sorting, provide expertise in implementing and developing technologies for cell analysis, and will provide training to investigators in the use of the instrumentation and in the interpretation of data analysis.

Expertise: The FACS Core Lab provides multiparameter flow cytometry and fluorescence-activated cell sorting on both live and fixed cells in suspension. Six fluorescent markers may be measured simultaneously at the single-cell level at a rate of 2000 cells per second. Protocols are available for detecting external, cytoplasmic, and nuclear antigens, DNA and cell cycle markers, and for doing functional assays.

Fluorescence-activated cell sorting allows two populations of cells to be simultaneously separated from the parent population. These sorted populations may be used for further cell culture or in biochemical or molecular assays. Rare cells can also be deposited directly into microtiter plates at the level of one cell per well for expansion of these populations.

The staff provides consultation for preparing samples for flow cytometry and setting up new experimental flow cytometry protocols, and trains investigators to use the instruments and analyze data.

Established Technologies:

Multiparameter (8 parameter, 6 color) fluorescent measurement and quantitation of cellular membrane, cytoplasmic, and nuclear antigens

Determination of cell cycle (GO/G1, S, G2/M phases) in fixed or live cells using fluorescent nucleic acid dyes and mathematical modeling; also measurement of specific cell cycle markers

Measurement of apoptosis on live or fixed cells

Detection of GFP and GFP variants (YFP, BFP, CFP)

Assays of cell function using fluorescent substrates

Sorting of cells based on antigen expression, cell cycle compartment, GFP expression.

Access

Open

Collaborations:

Adorjan Aszolas, LCB. Membrane potential, lipid packing, cell cycle analysis.

Mirit Aladjeem, LMP. Sorting of live cells by cell cycle.

Lena Diaw, LC. Sorting of live cells by surface antigen.

Nancy DiFronzo, Children's Research Institute, Washington, DC. Detection of surface antigens on fixed cells.

Publications:

DiFronzo NL, Leung CT, Georgopoulos K, Taylor BJ, Pham QP, Mammel MK. Ikaros binding to unique 3′ sequences of a mink-cell focusing virus is biologically significant. J. Virol. (accepted Oct. 2001).

EIB Flow Cytometry Facility

Experimental Immunology Branch (EIB)

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Experimental Immunology Branch, CCR

Web page: http://rex.nci.nih.gov/RESEARCH/basic/eib/sharrow.htm

Flow cytometric analyses and electronic cell separations

Mission: The mission of the Experimental Immunology Branch (EIB) Flow Cytometry Facility is to provide basic research support to members of the EIB.

Expertise: The facility operates and maintains two multi-laser research flow cytometers with high-speed electronic cell separation capabilities. Each of these instruments provides 10 detectors (8 fluorescence and 2 scatter) as well as electronic crossbeam compensation. The 3 lasers on each machine provide a range of excitation wavelengths from ultraviolet to red.

The facility also maintains 3 user-operated instruments providing single laser excitation at 488 nm and 3 fluorescence detectors.

The facility supports the EIB research program by performing flow cytometric analyses and electronic cell separations using multi-laser based instrumentation, and by providing and maintaining user-operated flow cytometers. Facility personnel also help EIB investigators with experimental design, selection of reagents, instrument operation, and data analysis.

The facility also provides consultation to laboratories outside the EIB, advanced training for operators of other flow cytometry facilities from the NIH and elsewhere, and limited flow cytometry support to other NCI laboratories.

Established Technologies:

Multi-color fluorescence single-cell-flow cytometric analyses

High-speed, high-purity cell sorting

Quantitative single-cell-fluorescence analysis

New Technologies Under Development:

Automated multi-parametric cluster analysis

Collaborations:

All flow cytometric work performed on the multi-laser instruments requires staff involvement and interaction with users.

Access

Lab/Program Dedicated

Support Staff

Lawrence Granger
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Anthony Adams, PhD
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Publications:

Cibotti R, Bhandoola A, Guinter TI, Sharrow SO, Singer A. CD8 coreceptor extinction in signaled CD4(+)CD8(+) thymocytes: Coordinate roles for both transcriptional and posttranscriptional regulatory mechanisms in developing thymocytes. Mol. Cell. Biol. 20:3852-3859, 2000.

Brugnera E, Bhandoola A, Cibotti R, Yu Q, Guinter TI, Yamashita Y, Sharrow SO, Singer A. Coreceptor reversal in the thymus: Signaled $CD4^+8^+$ thymocytes initially terminate CD8 transcription even when differentiating into $CD8^+$ T cells. Immunity. 13:59-71, 2000.

Bhandoola A, Kithiganahalli B, Granger L, Singer A. Programming for cytotoxic effector function occurs concomitantly with CD4 extinction during $CD8^+$ T-cell differentiation in the thymus. Intl. Immunol. 12:1035-1040, 2000.

Sheard MA, Sharrow SO, Takahama Y. Synchronous deletion of Mtv-superantigen-reactive thymocytes in the CD3 medium/high $CD4^+CD8^+$ subset. Scand. J. Immunol. 52:550-554, 2000.

McKean DJ, Huntoon CJ, Bell MP, Tai X, Sharrow SO, Hedin KE, Conley AA, Singer A. Maturation versus death of developing DP thymocytes reflects competing effects on bcl-2 expression and can be regulated by the intensity of CD28 costimulation. J. Immunol. 166:3468-3475, 2001.

Received Facility support:

Yu X, Fournier S, Allison JP, Sharpe AH, Hodes RJ. The role of B7 costimulation in CD4/CD8 T cell homeostasis. J. Immunol. 164:3543-3553, 2000.

Petiniot LK, Weaver Z, Barlow C, Shen R, Eckhaus M, Steinberg SM, Ried T, Wynshaw-Boris A, Hodes RJ. RAG-2 mediated V(D)J recombination is not essential for tumorigenesis in Atm-deficient mice. Proc. Natl. Acad. Sci. USA. 97:6664-6669, 2000.

Komoriya A, Packard BZ, Brown MJ, Wu M-L, Henkart PA. Assessment of caspase activities in intact apoptotic thymocytes using cell-permeable fluorogenic caspase substrates. J. Exp. Med. 191:1819-1828, 2000.

Lucas PJ, Kim SJ, Melby SJ, Gress RE. Disruption of T-cell homeostasis in mice expressing a T cell-specific dominant negative transforming growth factor beta II receptor. J. Exp. Med. 191:1187-1196, 2000.

Bodor J, Bodorova J, Gress RE. Suppression of T-cell function: A potential role for transcriptional repressor ICER. J. Leuk. Biol. 67:774-779, 2000.

Mackall CL, Stein D, Fleischer TA, Brown MR, Hakim FT, Bare CV, Leitman SF, Read EJ, Carter CS, Wexler LH, Gress RE. Prolonged CD4 depletion after sequential autologous peripheral blood progenitor cell infusions in children and young adults. Blood. 96:754-762, 2000.

Husebekk A, Fellowes V, Read EJ, Williams J, Petrus MJ, Gress RE, Fowler DH. Selection and expansion of T cells from untreated patients with CLL: Source of cells for immune reconstitution. Cytotherapy. 2:187-192, 2000.

Anderson HA, Hiltbold EM, Roche PA. Concentration of MHC class II molecules in lipid rafts facilitates antigen presentation. Nature Immunology. 1:156-162, 2000.

Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, Max EE, Ried T, Nussenzweig A. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. Nature. 404:510-514, 2000.

Chen HT, Bhandoola A, Difilippantonio MJ, Zhu J, Brown MJ, Tai X, Rogakou EP, Brotz TM, Bonner WM, Ried T, Nussenzweig A. Response to RAG-mediated V(D)J cleavage by NBS1 and gamma-H2AX. Science. 290:1962-1965, 2000.

Bhandoola A, Dolnick B, Fayad N, Nussenzweig A, Singer A. Immature thymocytes undergoing receptor rearrangements are resistant to an Atm-dependent death pathway activated in mature T cells by double-stranded DNA breaks. J. Exp. Med. 192:891-898, 2000.

Bosselut R, Kubo S, Guinter T, Kopacz JL, Altman JD, Feigenbaum L, Singer A. Role of CD8b domains in CD8 coreceptor function: Importance for MHC-I binding, signaling, and positive selection of $CD8^+$ T cells in the thymus. Immunity 12:409-418, 2000.

Visintin A, Mazzoni A, Spitzer JH, Wyllie DH, Dower SK. Segal DM. Regulation of toll-like receptors in human monocytes and dendritic cells. J. Immunol. 166:249-255, 2001.

Fry TJ, Christeensen BL, Komschlies KL, Gress RE, Mackall CL. IL-7 restores immunity in athymic T cell depleted hosts. Blood in press.

Mackall CL, Fry TJ, Bare C, Morgan P, Galbraith A, Gress RE. IL-7 increases both thymic-dependent and thymic-independent T-cell regeneration. Blood. (in press).

Haddad EK, Wu Z, Hammer JA III, Henkart PA. Defective granule exocytosis in Rab27a-deficient lymphocytes from ashen mice. J. Cell Biol. 152:835-842, 2001.

Frederick CCR Flow Cytometry Core

Laboratory of Experimental Immunology (LEI)

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Laboratory of Experimental Immunology, CCR, NCI-Frederick

Comprehensive support for studies of cell cycle, apoptosis, and cell ploidy

Mission: The CCR Flow Cytometry Core provides NCI and other organizations with state-of-the-art flow cytometry analysis and cell sorting of cell suspensions derived from various tissues and cultured cells. This group provides support for comprehensive basic research studies on the cell cycle, apoptosis, cell ploidy, sterile cell sorts, single cell cloning and development of new technologies. This laboratory is contract-operated with direction provided by Dr. John Ortaldo.

Expertise: The CCR Flow Cytometry Core provides multi-color flow cytometry analysis of live or fixed single-cell suspensions derived from various tissues and cell cultures. The group can offer up to four parameter cell surface markers coupled with calcium-flux or cell-cycle analysis.

Cell sorting of single-cell suspensions of up to three colors is available in either bulk sorts or by single cell deposition in 96-well plates.

Cell cycle analysis software is available for cell ploidy, cell cycle and apoptotic analysis of cell populations.

Established Technologies:

- Immunophenotyping with four-color analysis
- Cell-cycle and cell-ploidy analysis
- Sterile cell sorting and single-cell cloning
- Calcium mobilization analysis
- Experience with multiple fluorochromes, including GFP and β-gal detection
- Apoptosis analysis
- Immunophenotyping of transgenic/knockout mice

New Technologies Under Development: Discovering the strengths and weaknesses of the new three laser LSR cytometer from Becton Dickinson. The laser wavelengths and configuration of the machine optics provide for opportunities for combining DNA dyes and calcium flux dyes with four-color immunophenotyping of cell populations.

Access

Lab/Program Dedicated

Support Staff

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Instrumentation:

Becton Dickinson LSR: A bench top analyzer with Argon, HeNe and HeCad lasers and 8 acquisition parameters

Becton Dickinson FACScan: A bench top analyzer with an Argon laser and up to three-color analysis

Becton Dickinson FACSTAR^{PLUS}: A conventional sorter with two Argon lasers, one of which has UV capability

Collaborations:

Part of the job of the group is to give advice and experimental design suggestions to the investigators who use this facility. This advice includes trouble-shooting when results are not as good as they could be. We keep staining protocols on file which we provide when an investigator is trying a new procedure.

Publications:

Neubauer RH, Briggs CJ, Noer KB, Rabin H. Identification of normal and transformed lymphocyte subsets of nonhuman primates with monoclonal antibodies to human lymphocytes. J. Immunol. 130:1323-1329, 1983.

Leonard EJ, Noer K, Skeel A. Analysis of human monocyte chemoattractant binding by flow cytometry. J. Leukoc. Biol. 38:403-413, 1985.

Leonard EJ, Skeel A, Yoshimura T, Noer K, Kutvirt S, Van Epps D. Leukocyte specificity and binding of human neutrophil attractant/activation protein-1. J. Immunol. 144:1323-1330, 1990.

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NCI ETIB Flow Cytometry Core Laboratory

Experimental Transplantation and Immunology Branch (ETIB)

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Experimental Transplantation and Immunology Branch, CCR

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Advanced high-speed cell sorting, multicolor analysis and laser scanning cytometry

Mission: The NCI Experimental Transplantation and Immunology Branch Flow Cytometry Core Laboratory provides custom research flow and image cytometry services to all investigators in the NCI Experimental Transplantation and Immunology Branch (ETIB) and other NIH and outside investigators. The Core offers advanced high-speed cell sorting, complex multicolor analysis and laser scanning image cytometry services, as well as support for routine benchtop flow cytometric analysis. The Core provides support for flow cytometric technique development by Branch users, and maintains its own internal research program for assay and instrument technology development.

Expertise: The Core provides support in flow cytometry technique development to Branch investigators. This includes assisting investigators in integrating existing flow cytometric technology into their research projects, and collaborating with Branch researchers to develop novel techniques to solve specific research problems. The Core is an active contributor to branch scientific research efforts as well as a service provider.

The Core additionally maintains an internal development program aimed at advancing flow cytometric technology, particularly in areas of advanced cell analysis, such as multicolor multilaser flow cytometry and laser scanning cytometry.

Established Technologies:

- High-speed cell sorting (including sterile and rare event sorting
- Complex multicolor flow cytometric analysis (up to six fluorescent colors simultaneously)
- Flow cytometric analysis using unusual laser sources
- Routine multicolor flow cytometric analysis (up to four colors) using benchtop flow cytometers
- Laser scanning (image) cytometry
- Digital fluorescence microscopy.

New Technologies Under Development:

- Identification of novel fluorochromes for flow cytometry (including newly identified phycobiliproteins, intact phycobilisomes and

Access

Lab/Program Dedicated

Support Staff

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fluorogenic enzyme substrates)

Application of recently developed low-power laser sources for flow cytometry

Characterization of novel fluorescent probes for analysis of cell physiology.

Collaborations:

Dan Fowler, ETIB, CCR, NCI. Analysis of CTL-mediated cytolysis by laser scanning cytometry using fluorogenic caspase substrates.

Susan Bates, CTB, CCR, NCI. Characterization of MXR membrane pump substrate anticancer drugs for fluorescence properties.

Gerald Marti, CBER FDA, NIH. High-resolution analysis of CLL hypodiploidy by flow and image analysis.

Carl Feng, Laboratory of Parasitic Diseases, NIAID. Lymphocyte immunophenotyping by laser scanning cytometer in lung tissue sections.

Teresa Hawley and Bob Hawley, Hematopoiesis Section, American Red Cross Holland Laboratory. Multicolor flow analysis of expressible fluorescence proteins.

Stephen Doty, Mineralized Tissue Section, Hospital for Special Surgery-Weill-Cornell University School of Medicine, NY, NY. Random contouring methodologies for tissue section analysis by laser scanning cytometry.

Alexander Sapozhnikov, Sheyakin and Ovchinnikov Institute for Bioorganic Chemistry, Moscow, Russia. Development of techniques for detection of intracellular antigens, particular heat shock proteins.

Publications:

Maki W, Telford WG, Knibbs RN, Stoolman LM, Hwang ST. CCR6 co-localizes with CD18 and enhances adhesion to activated endothelial cells in CCR6-transduced Jurkat T cells. Submitted.

Telford WG, Komoriya A, Packard BZ. Detection of localized caspase activity in early apoptotic cells by laser scanning cytometry. Submitted.

Ellis CA, Wickline M, Riley C, Telford WG, Vos M, Zujewski J, Clark GJ. Tamoxifen and the farnesyl transferase inhibitor FTI-277 synergize to inhibit growth in estrogen receptor-positive breast tumor cell lines. Submitted.

Yamada N, Tagaya Y, Telford WG, Katz SI. Expression of natural killer cell markers on mast cells. Submitted.

Telford WG, Moss MM, Morseman JP, Allnutt FCT. Cyanobacterial stabilized phycobilisomes as fluorochromes for extracellular antigen detection by flow cytometry. J. Immunol. Methods. 254:13-30, 2001.

Hawley TS, Telford WG, Ramezani A, Hawley RG. Four-color flow cytometric detection of retrovirally expressed cyan, green, yellow and red fluorescent proteins. BioTechniques 30:1028-1034, 2001

Telford WG, Moss MM, Morseman JP, Allnutt FCT. Cryptomonad algal phycobiliproteins as fluorochromes for extracellular and intracellular antigen detection by flow cytometry. Cytometry 44:16-23, 2001.

Hawley TS, Telford WG, Ramezani A, Hawley RG. "Rainbow" reporters for multispectral marking and lineage analysis of hematopoietic stem cells (review article). Stem Cell 19:118-124, 2001.

Telford WG, Cox WG, Singer VL. Detection of endogenous and antibody-conjugated alkaline phosphatase with ELF-97 phosphate in multicolor flow cytometry applications. Cytometry 43:117-125, 2001.

Hematopathology Section

Laboratory of Pathology (LP)

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Diagnostics, education and research integrating histochemistry and molecular diagnostics

Mission: The mission of the Hematopathology Section is to achieve the highest level of quality in diagnostics, education and research in partnership with the clinical and research community of the National Institutes of Health. By continuing to expand the scope of our expertise and by incorporating the newest tools of diagnosis, training, and research, our goal is to be a globally recognized center of excellence for clinical diagnosis, disease research, and pathology education in hematopathology.

Expertise: The staff of the Hematopathology Section provides expertise in the diagnosis of lymphoma and other lymphoproliferative lesions. We also provide diagnostic services in in-situ hybridization, and with the core services of LP, integrate immunohistochemistry and molecular diagnostics into the diagnostic report. Services are provided to the staff of the NIH community, as well as to physicians and other pathologists, both nationally and internationally. Approximately 1800 cases are reviewed annually. Areas of emphasis include:

B-cell lymphomas

T-cell lymphomas

Hodgkin's lymphoma

Atypical reactive hyperplasias

Autoimmune lymphoproliferative syndrome

Autoimmune disease

Lymphomatoid granulomatosis and other EBV-related lympho proliferative disorders

Post-transplant lymphoproliferative diseases.

Established Technologies:

Histopathological diagnosis

Immunohistochemistry on frozen and paraffin sections

In-situ hybridization for detection of EBV and other potential targets

Access

Lab/Program Dedicated

Support Staff

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Collaborations:

Dr. Wyndham Wilson, Medicine Branch, DCS, NCI. Lymphoma specimens for diagnosis and immunophenotyping.

Dr. Richard Little, HAMB, NCI. Lymphoma specimens for diagnosis and immunophenotyping.

Dr. Stephen Straus, NIAID. Lymph nodes for diagnosis and immunophenotyping.

Dr. Giovanna Tosato, Medicine Branch, NCI. Tissue specimens for diagnosis, immunophenotyping and in-situ hybridization.

Dr. Alan Wayne, POB, DCS, NCI. Lymphoma specimens for diagnosis and immunophenotyping.

Dr. Robert Kreitman, NCI. Lymphoma specimens for diagnosis and immunophenotyping.

Dr. Thomas Waldmann, Metabolism Branch, DCS, NCI. Lymphoma specimens for diagnosis and immunophenotyping.

Dr. David Nelson, Metabolism Branch, DCS, NCI. Lymphoma specimens for diagnosis and immunophenotyping.

Publications:

Yao X, Teruya-Feldstein J, Raffeld M, Sorbara L, Jaffe ES. Peripheral T-cell lymphoma with aberrant expression of CD79a and CD20: A diagnostic pitfall. Mod. Pathol. 14:105-110, 2001.

Gutiérrez MI, Kingma DW, Sorbara L, Tran M, Raffeld M, Jaffe ES, Magrath I, Bhatia K. Association of EBV strains, defined by multiple loci analyses, in non-Hodgkin lymphomas and reactive tissues from HIV-positive and HIV-negative patients. Leuk. Lymphoma 37:425-429, 2000.

Longo DL, Duffey PL, Gribben JG, Jaffe ES, Curti BD, Gause BL, Janik JE, Bramen VM, Esseltine D, Wilson WH, Kaufman D, Wittes RE, Nadler LM, Urba WJ. Combination chemotherapy followed by a recombinant immunotoxin (anti-B4-blocked ricin) in patients with indolent lymphoma: Results of a phase II study. Cancer J. Sci. Am. 6:146-150, 2000.

Little RF, Gutierrez M, Jaffe ES, Pau A, Horne M, Masur H, Wilson WH. HIV-associated Non-Hodgkin's lymphoma: Incidence, presentation, and prognosis. JAMA. 285:1880-1885, 2001.

Lopatin U, Yao X, Williams RK, Dale JK, Wong D, Teruya-Feldstein J, Fritz S, Fuss I, Sneller MC, Raffeld M, Puck JM, Strober W, Jaffe ES, Straus SE. Increases in circulating and lymphoid tissue IL-10 in autoimmune lymphoproliferative syndrome (ALPS) are associated with disease expression. Blood 97:3161-70, 2001.

Lei JY, Wang Y, Jaffe ES, Turner ML, Raffeld M, Sorbara L, Morris J, Holland SM, Duray PH. Microcystic adnexal carcinoma associated with primary immunodeficiency, recurrent diffuse herpes simplex virus infection, and cutaneous T-cell lymphoma. Am. J. Dermatopathol. 22:524-529, 2000.

Straus SE, Jaffe ES, Puck JM, Dale JK, Elkon KB, Rosen-Wolff A, Peters AMJ, Sneller MC, Hallahan CW, Wang J, Fischer RE, Jackson CM, Lin AY, Baumler C, Siegert E, Marx A, Vaishnaw AK Grodzicky T, Fleisher TA, Lenardo MJ.

The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. Blood, in press.

Beaty MW, Toro J, Sorbara L, Stern JB, Pittaluga S, Raffeld M, Wilson W, Jaffe ES. Cutaneous lymphomatoid granulomatosis: Correlation of clinical and biological features. Am. J. Surg. Pathol., in press.

Wilson WH, O'Connor P, Jaffe ES, Chabner BA, Grossbard M. Role of rituximab and chemotherapy in aggressive B-cell lymphoma: A preliminary report of dose-adjusted EPOCH-R, Seminars in Oncology, in press.

Sarkodee-Adoo C, Pittarelli L, Jaffe ES, Yao X, Raffeld M, Heller T. Regression and clonally distinct recurrence of human immunodeficiency virus-related Burkitt-like lymphoma during antiretroviral therapy. Leuk. Lymphoma, in press.

Robinson MR, Salit RB, Bryant-Greenwood PK, Zeichner SL, Wood LV, Jaffe ES, Van Waes C, Magrath IA. Burkitt's/Burkitt's-like lymphoma presenting as bacterial sinusitis in HIV-infected children. AIDS Patient Care and STDs, in press.

Harty LC, Lin AY, Goldstein AM, Jaffe ES, Carrington M, Tucker MA, Modi WS. HLA-DR, HLA-DQ, and TAP genes in familial Hodgkin's disease. Blood, in press.

Teruya-Feldstein J, Kingma DW, Weiss A, Sorbara L, Burd PR, Raffeld M, Mueller BU, Tosato G, Jaffe ES. Chemokine gene expression and clonal analysis of HIV-associated lymphoid interstitial pneumonitis in pediatric patients. Mod. Pathol., in press.

Clinical Services Program
(CSP)

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Mission: The primary mission of the Clinical Services Program (CSP) is to perform sequential studies of immune function in patients with cancer, AIDS, chronic granulomatous disease (CGD) or chronic fatigue syndrome (CFS) during treatment with biological response modifiers or other potential anticancer or antiviral agents. Alterations in immune function are correlated with the patient's clinical response to treatment, and are used to design second-generation clinical studies and guide basic research into the agent's mechanisms of action.

Expertise: The CSP was established in late 1981 to provide dedicated laboratory facilities to the Biological Response Modifiers Program, National Cancer Institute. During the next decade, the CSP was expanded to provide a full range of dedicated laboratory and clinical facilities to the Medicine Branch, HIV Adult Malignancy Branch, and Surgery Branch, Division of Clinical Sciences, the Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, the Biological Resources Branch, Division of Cancer Treatment and Diagnosis, the Laboratories of Immunoregulation, Clinical Investigations and Host Defenses and the Division of Intramural Research, National Institute of Allergy and Infectious Disease.

The organization of the CSP has evolved and expanded over the last 19 years, so that each laboratory/administrative component is structured to adapt to specific technical workscope requirements in support of the various government programs. A highly trained scientific staff, a centralized administrative and computer programming support staff, and state-of-the art laboratories and clinical facilities allow for comprehensive immunological monitoring and research programs in support of diverse clinical trials. The CSP comprises 13 individual laboratories and a centralized administrative group, with each laboratory responsible for defined areas of the workscope. Technical staff have been cross-trained in a wide variety of immunological procedures, and equipment resources are shared, where appropriate, resulting in the most efficient use of labor and resources. The CSP has gained a reputation as a benchmark laboratory for this type of work.

Established Technologies:

Two-, three-, and four-color flow cytometric phenotypic analysis of peripheral blood, mononuclear cells and cell lines

Access

Lab/Program Dedicated

Support Staff

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Two- and three-color cell sorting using flow cytometry

Lymphokine and cytokine testing

Blast transformation assays

Cytotoxic assays

Viral burden assays (quantitative PCR, bDNA, NASBA, p24 antigen and RT PCR)

Quantitation of stem cells from peripheral blood and bone marrow samples

Production of activated NK and T cells ex vivo for reinfusion into patients

Complete assessments of granulocyte and monocyte function.

New Technologies Under Development:

ELISPOT

DNA Microarray technologies

Publications:

Derby EG, Reddy V, Nelson EL, Kopp WC, Baseler MW, Dawson JR, Malyguine AM. Correlation of human $CD56^+$ cell cytotoxicity and IFN-γ production. Cytokine. 13(2):85-90, 2001.

Gea-Banacloche JC, Martino L, Mican JM, Hallahan CW, Baseler M, Stevens R, Lambert L, Polis M, Lane HC, Connors M. Longitudinal changes in $CD4^+$ T-cell antigen receptor diversity and naive/memory cell phenotype during 9 to 26 months of antiretroviral therapy of HIV-infected patients. AIDS Res. Hum. Retroviruses. 16(17):1877-1886, 2000.

Lempicki RA, Kovacs JA, Baseler MW, Adelsberger JW, Dewar RL, Natarajan V, Bosche MC, Metcalf JA, Stevens RA, Lambert LA, Alvord WG, Polis MA, Davey RT, Dimitrov DS, Lane HC. Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of $CD4^+$ and $CD8^+$ T-cell turnover in HIV-infected patients. Proc. Natl. Acad. Sci. (USA). 97(25):13778-13783, 2000.

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Tissue Array Research Program
(TARP)

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Microarrays from 500 tissues or tumors

Mission: The Tissue Array Research Program (TARP) is a collaborative effort between The National Cancer Institute and The National Human Genome Research Institute. The primary objective of TARP is to develop and disseminate Multi-Tumor Tissue Microarray slides and the related technology to the cancer research investigators. This enabling technology will help expedite discovery of novel targets important in cancer treatment by providing a tool for high-throughput screening of multiple tumor tissues using immunohistochemical, in situ, and FISH analyses. Secondary goals include the further development of tissue microarray technology, investigations into methods that may be used to probe tissue microarrays, and dissemination of information about microarray construction and use.

Expertise: Our core is engaged in the construction of tissue microarrays. Tissue microarrays are arrays of formalin-fixed tissues embedded in paraffin blocks. A single microscope slide cut from such a block may show tissues from 100 to 600 separate individuals. Because large numbers of tissues can be placed on a single slide, tissue microarrays are ideal tools for tissue surveys looking at incidence or prevalence of proteins or transcripts in particular tumors or tissues.

The core is currently used to construct two types of arrays. Our main focus is the ongoing production of a multi-tumor array containing 25 to 75 cases of 8 different malignancies, plus 50 normal tissue controls. Tumors represented in this array include carcinomas from breast, prostate, lung, colon, and ovary, as well as melanoma, lymphoma and glioblastoma. A total of 500 tissues and tumors are arrayed. All of these tissue have been anonymized. Slides prepared from these arrays may be obtained through the Cooperative Human Tissue Network. A secondary focus is the collaborative development of specialty arrays. These arrays make use of tissues and tumors that are linked to more detailed clinical information. The use of these arrays is subject to the IRB-approved protocol under which the tissues were obtained.

Established Technologies: Standard histology techniques, including

Tissue fixation and processing

Paraffin embedding of tissues

Cutting sections and hematoxylin and eosin staining

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Cutting sections using the tape sectioning methods which reduce distortion of the tissues during transfer to the microscope slide

Immunohistochemical staining of tissue microarray slides

Tissue microarray construction using a manual microarray tool

Construction of paraffin embedded cell blocks.

New Technologies Under Development:

Tissue microarray construction using an automated tissue microarray tool

Digital imaging of tissue microarrays for automated or manual interpretation.

Collaborations:

Dr. Marston Linehan, CCR, UOB, NCI. Kidney tumor array.

Dr. Lee Helman, CCR, POB, NCI. Dog osteosarcoma array.

Jin Jen, LPG, DCEG, NCI. Non-small cell lung carcinoma array.

Dr. Kevin Gardner, LP, CCR, NCI. Role of p300 and BRCA1 in cancer using tumor model system arrays.

Dr. Kevin Gardner and Dr. Michael Emmert-Buck, LP, CCR, NCI. Technology development in tissue microarray use.

Dr. John Weinstein, LMP, NCI. Construction and analysis of tissue microarrays based on the NCI-60 cell lines.

Publications:

Hewitt SM, Kleiner DE. Tissue arrays, high-throughput technology comes to pathology. BioLink, in press.

Laser Capture Microdissection Core Facility
(Laboratory of Pathology)

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Laser capture microdissection of tissue to isolate pure cell populations

Mission: The Laser Capture Microdissection Core Facility provides cutting-edge technology that allows NIH researchers means to obtain pure populations of cells from heterogeneous pieces of tissue.

Expertise: The facility provides three PixCell II laser-capture microdissection instruments for the procurement of pure cell populations from heterogeneous tissue sections to be further analyzed by various downstream tests using the RNA, DNA or proteins extracted from the cells captured by LCM. Two of the systems are equipped with fluorescence, enabling the researcher to use immunofluorescence as a means of identifying specific cells. The core facility also provides staining facilities for standard hematoxylin and eosin (H&E) staining of tissue, as well as a Tissue Tek 2000 cryostat for the preparation of tissue sections for microdissection.

Training is provided for the use of the PixCell II instruments, cyrostat and staining techniques compatible with laser capture microdissection on a one-to-one basis. Ongoing technical support is available for researchers from the core staff. This training and support are available to all institutes within NIH, as well as researchers from other research institutions around the country and the world.

Established Technologies: Laser-capture microdissection with light and fluorescent visualization are available. Tissue sample can be used for various downstream testing such as PCR, LOH, Western blotting, 2D gel electrophoresis, cDNA arrays, etc.

Tissue section preparation for microdissection is made possible by a Tissue Tek 2000 cryostat for the sectioning of tissue and H&E for staining of slides.

Publications:

Pawelez CP, Charboneau L, Bichsel VE, Simone NL, Chen T, Gillespie JW, Emmert-Buck MR, Roth MJ, Petricoin EF 3rd, Liotta LA. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene. 20:1981-1989, 2001.

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Image Analysis Laboratory (IAL)

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Multi-color, high-resolution, fluorescence images of fixed and live specimens

Mission: The Image Analysis Laboratory (IAL) provides NCI and other investigators with state-of-the-art 3D optical (confocal) and electron microscopy facilities for imaging of living and fixed cells and tissues; computational resources for visualization and extraction of quantitative information from images; and develops new technologies for cutting-edge cancer research.

Expertise: The Confocal Microscopy Facility provides multi-color, high-resolution fluorescence image acquisition of fixed and live specimens (up to three colors). Specimens can be up to 50 microns thick.

Microscopy of live cells includes measurement of molecular dynamics.

Image Analysis software is available for segmenting (identifying) cells and subcellular components (e.g., cell nuclei) from the images of specimens and for quantifying their structural properties (e.g., size and shape) and their molecular properties (e.g., amount of a fluorescence signal).

The Electron Microscopy Facility offers negative-stained and thin-sectioned ultrastructural analysis with transmission electron microscopes (TEM) and three-dimensional ultrastructural image analysis with a scanning electron microscope (SEM). The group offers immunoelectron microscopy (IEM) for pre- and postembedding, or negatively stained IEM. Also available are several types of light microscopy (LM): stereo microscopy, inverted microscopy, and fluorescent microscopy.

The staff of the IAL are experts in computer programming, quantitative image analysis, optical microscopy, electron microscopy and techniques for specimen preparation.

Established Technologies: Images acquired by either confocal or electron microscopy are automatically archived to the Advanced Biomedical Computing Center (ABCC) and are always available to users via the Internet.

The Confocal Microscopy Facility offers the following techniques:

- Multi-color, high-resolution fluorescence image acquisition of fixed and live specimens (up to three colors)
- Bright-field, phase and differential interference contrast (Nomarsky) imaging in conjunction with fluorescence imaging

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Time-lapse imaging of live cells

Ratio imaging of calcium ions and other elements

Experiments involving the uncaging of molecules in cells

Fluorescence Resonance Energy Transfer (FRET) experiments (FRET is a technique to detect the binding of two fluorescence-tagged molecules in cells.)

Fluorescence Recovery After Photobleaching (FRAP) experiments (FRAP is a technique to measure the diffusion of fluorescence-tagged molecules, by photobleaching the fluorescent molecules in a defined region of the cell and then observing the recovery of fluorescence into the same region from the unbleached surroundings.)

Image Analysis software for segmenting (identifying) cells and sub-cellular components (e.g., cell nuclei) from the images of specimens and for quantifying their structural properties (e.g., size and shape) and their molecular properties (e.g., amount of a fluorescence signal).

Software is also available for measuring the cellular organization of tissue and the degree of co-localization of two fluorescence-tagged molecular species in cells.

Transmission Electron Microscopy (TEM) and Immunoelectron Microscopy (IEM):

Thin-sectioned TEM ultrastructural analysis of tissues and cells

Semiquantitative TEM analysis of virus pellet samples

Postembedding IEM analysis of tissues and cells

Silver-enhanced IEM analysis of tissues and cells

Negative-stained TEM ultrastructural and semiquantitative analysis of bacteria, protein, and virus samples

Negative-stained IEM analysis of bacteria and virus samples

Shadow replica TEM analysis of virus and virus core samples

TEM analysis of DNA, DNA-protein complexes, and RNA samples

TEM analysis of heteroduplex between DNA-DNA and DNA-RNA samples

TEM analysis of whole-chromosome mount

TEM autoradiography

Scanning Electron Microscopy (SEM)

SEM analysis of cultured-cell and tissue samples

SEM analysis of cryofractured tissue samples

SEM analysis of cell-surface immunolabeling

Light Microscopy (LM) and Immunofluorescence Microscopy (IFA)

Semi-thin-sectioned LM macro analysis of tissues from resin-embedded block

LM analysis of cells or tissues by in-situ hybridization (3H or 35S)

LM autoradiography

IFA analysis of cultured cell or tissue samples.

New Technologies Under Development:

Image Access: Software for image archive, image processing and analysis and generation of presentations. Currently being tested by the IAL before release to users at NCI-Frederick.

Software to measure protein internalization from the cell surface to the cytoplasm, and to quantify the level of colocalization of two proteins on the cell surface or in the cytoplasm.

Modeling of protein trafficking between the nucleus and cytoplasm.

Software to measure gene expression at the individual cell level within intact tissue.

Quantification of protein concentrations and co-localization using immunogold labeling and transmitted electron microscopy.

Silver-enhancement of immunogold labels so they can be visualized both by optical and confocal microscopy.

Collaborations:

Confocal Microscopy:

Dr. Colin Stewart, CDBL, NCI-Frederick. 3D image analysis software to analyze nuclear size, shape and organization in tissue.

Dr. Ji Ming Wang, LMI, NCI-Frederick, and Dr. Howard Young, NCI-Frederick. Quantitative analysis of protein localization and protein-protein interactions.

Dr. George Pavlakis, BRL, NCI-Frederick. Quantitative analysis and computer modeling of protein trafficking using fluorescence recovery after photobleaching.

Dr. Robert Wiltrout, LEI, NCI-Frederick. Quantitative analysis of tissue structure.

Dr. Stan Burt, ABCC, SAIC, NCI-Frederick. Computer support (hardware and software) for data backups and image analysis development.

Jim McNally, NCI-Bethesda. Image Access (see above).

Matthew McAuliffe, CIT-Bethesda. Freely available image analysis software for users.

Electron Microscopy:

A.O. Williams, DCRDP, ORM, CDER, FDA. Thick and thin section TEM of mouse tumor tissue.

Dr. Mariana Gerschenson, DHVD, NHLBI, NIH. TEM analysis of drug-treated monkey tissue.

Dr. Robert Wiltrout and Dr. Jon Wigginton, LEI, CCR, NCI-Frederick. Thick and thin-section analysis of mouse kidney tumor.

Dr. Kazmierz Kasprzak, LCC, CCR, NCI-Frederick. TEM and IEM analysis of rat tissue.

Dr. S. Kimura, LM, CCR, NCI. TEM and IEM of transgenic mouse tissue. TEM freeze fracture study of cell junction.

Publications:

Sarti A, Ortiz de Solorzano C, Lockett S, Malladi R. Computer-aided cytology: A geometric model for 3D confocal image analysis. IEEE Trans. Biomed. Eng. 47(12):1600-1609, 2000.

Lieb JD, Ortiz de Solorzano C, Rodriguez EG, Jones A, Angelo M, Lockett S, Meyer BJ. The *Caenorhabditis elegans* dosage compensation machinery is recruited to X chromosome DNA attached to an autosome. Genetics. 156:1603-1621, 2000.

Ortiz de Solorzano C, Malladi R, Lelièvre SA, Lockett SJ. Segmentation of nuclei and cells using membrane-related protein markers. J. Microsc. 201(1):1-13, in press 2001.

CCR Confocal Microscopy Core

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High resolution microscopic analyses of live and fixed specimens

Mission: The Confocal Microscopy Core Facility provides state-of-the-art histologic and microscopic analyses to better understand critical biological structures and cellular processes involved in cancer. Our goal is to make readily available to NCI investigators state-of-the-art equipment and training for acquisition of images from both fixed and living cells and tissue sections. The facility also provides post-acquisition processing of these images so that users can view or merge images, make measurements, 3D reconstructions, time-lapse movies and publication-quality prints.

Expertise: The facility provides expertise in microscopy, sample preparation, tissue culture, immunohistochemistry and image acquisition and analysis.

Imaging of live cells with a temperature-controlled, perfuseable, enclosed chamber, as well as imaging of fixed specimens is available so that 3D tomography of biological specimens or time-lapse studies of cellular processes can be performed. Time-lapse studies have been combined with 3D tomography to produce 4D acquisition data.

Established Technologies: Some of the imaging techniques that have been used in the Confocal Microscopy Core Facility are:

- Multi-labeling with green, red and far-red fluorescence (FITC-phalloidin for for actin, Rhodamine for tubulin, ToPro-3 for DNA) in fixed cells or sections
- Propidium iodide staining for apoptosis in fixed cells and sections
- Co-localization of Green Fluorescent Protein (GFP) fusion proteins with organelles (mitochondria, endoplasmic reticulum, lysosomes, Golgi, and endosomes)
- Measurement of oxidative stress using fluorescent indicators in live cells
- Cytoskeleton and membrane ruffling in fixed lymphoid cells
- Live-cell imaging with GFP to study protein activation and translocation over time in response to drugs or chemicals
- Relationship between HMG proteins and chromatin structure using triple labeling.

Time-lapse studies have been combined with 3D tomography to produce 4D acquisition data.

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New Technologies Under Development: An additional confocal system was purchased for the facility at the end of FY 2000 and is now available to users. This system is the Zeiss LSM 510 with visible Argon, HeNe, and UV Argon lasers and an inverted Axiovert microscope. Fluorescent Recovery after Photobleaching (FRAP) and Fluorescence Resonance Energy Transfer (FRET) experiments, as well as imaging of UV-excitable dyes, can be conducted with the new system. FRAP methodology involves time-lapse imaging with high illumination levels to bleach green fluorescent protein (GFP) fusion proteins so that analysis of the movement of unbleached GFP chimeras into bleached areas can be observed and quantitated over extended periods of time. An additional computer and Zeiss software have been purchased to provide an independent processing station for the new system.

Image Access, an image database software, has been recently purchased to serve as an image management system for archiving, browsing, searching, retrieving, sorting, printing and producing reports. This image database will accommodate several types of image data, including digital photographs, 2D microscope images, 3D confocal images, gels, phosphor imager data, flow cytometry histograms, microarray data, manuscript figures, slides, and Powerpoint presentations. Once the database is fully operational, users will have access to and will be trained in using this image database.

Collaborations:

Dr. Zsolt Jobbagy and Dr. Wayne Anderson, DCBDC, NCI. Subcellular redistribution of Pit-2 Pi transporter.

Dr. Peter Blumberg, DCEG, NCI; Dr. Patricia Lorenzo, DCEG, NCI; Qiming Wang, DCEG, NCI. PKC translocation in living cells.

Marta Bosak and Dr. Michael Bustin, DCEG, NCI. Chromatin and HMG proteins.

Ester Fernandez, Kwang Suh and Dr. Stuart Yuspa, DCEG, NCI. mtclic localization and function.

Marianna Mertts and Slava Tomarev, NEI, NIH. Cytoplasmic localization of Myoc/Tigr and its association with microtubules.

Publications:

Tomizawa M, Garfield S, Factor V, Xanthopoulos KG. Hepatocytes deficient in CCAAT/enhancer binding protein (C/EBP) exhibit both hepatocyte and biliary epithelial cell character. Biochem. Biophys. Res. Commun. 249: 1-5, 1998.

Conner EA, Teramoto T, Wirth PJ, Kiss A, Garfield S, Thorgeirsson SS. HGF-mediated apoptosis via p53/bax independent pathway activating JNK1. Carcinogenesis 20:583-590, 1999.

Ritter LM, Garfield SH, Thorgeirsson UP. A chimeric protein of tissue inhibitor of metalloproteinases-1 and the enhanced green fluorescent protein can be used to visualize the presence of cell surface receptors. Biochem. Biophys. Res. Commun. 257:494-499, 1999.

Romanova LY, Alexandrov IA, Blagosklonny MV, Nordan RP, Garfield S, Acs P, Nguyen P, Trepel J, Blumberg PM, Mushinki JF. Regulation of actin cytoskeleton in lymphocytes: PKC-disrupts IL-3-induced membrane ruffles downstream of Rac1. J. Cell Physiol. 179:157-169, 1999.

Lorenzo PS, Bogi K, Hughes KM, Beheshti M, Bhattacharyya D, Garfield SH, Pettit GR, Blumberg PM. Differential roles of the tandem C1 domains of protein kinase C delta in the biphasic down-regulation induced by bryostatin *1.* Cancer Res. 59:6137-6144, 1999.

Mertts M, Garfield S, Tanemoto GK, Tomarev SI. Identification of the region in the N-terminal domain responsible for the cytoplasmic localization of Myoc/Tigr and its association with microtubules. Lab. Investig. 79:1237-1245, 1999.

Wang QJ, Bhattacharyya D, Garfield S, Nacro K, Marquez VE, Blumberg PM. Differential localization of protein kinase C delta by phorbol esters and related compounds using a fusion protein with green fluorescent protein. J. Biol. Chem. 274:37233-37239, 1999.

Song S, Grenfel TZ, Garfield S, Erikson RL, Lee KS. Essential function of the polo-box of Cdc5 in subcellular localization and induction of cytokinetic structure. Mol. Cell. Biol. 20:286-298, 2000.

Jobbagy Z, Garfield S, Baptiste L, Eiden MV, Anderson WB. Subcellular redistribution of Pit-2 P(i) transporter/amphotropic leukemia virus (A-MuLV) receptor in A-MuLV-infected NIH 3T3 fibroblasts: involvement in superinfection interference. J. Virol. 74:2847-2854, 2000.

Wang QJ, Fang T, Fenick D, Garfield S, Bienfait B, Marquez VE, Blumberg PM. The lipophilicity of phorbol esters as a critical factor in determining the pattern of translocation of protein kinase C delta fused to a green fluorescent protein. J. Biol. Chem. 275:12136-12146, 2000.

Kronfeld I, Kazimirsky G, Lorenzo PS, Acs P, Garfield SH, Blumberg PM, Brodie C. Phosphorylation of protein kinase C delta on distinct tyrosine residues regulates specific cellular functions. J. Biol. Chem. 275:35491-35498, 2000.

Nacro K, Sigano DM, Pearce LL, Lewin NE, Garfield SH, Blumberg PM, Marquez VE. A potent protein kinase C activator diacylglycerol combines the highest binding affinity (KI) with the lowest liphophilicity. J. Med. Chem., 2000.

Lorenzo PS, Kung JW, Bottorff DA, Garfield SH, Stone JC, Blumberg PM. Phorbol Esters modulate the Ras exchange factor Ras GRP3. Cancer Res. 61:943-949, 2001.

Prymakowksa-Bosak M, Mistel T, Herrera JE, Shirakawa H, Birger Y, Garfield SH, Bustin M. Mitotic phosphorylation prevents the binding of HMGN + proteins to chromatin. Mol. Cell. Biol., 2001.

Wang, XW, Tseng A, Ellis NA, Spillare EA, Linke SP, Robles AL, Seker H, Yang Q, Hu P, Beresten S, Bemmels NA, Garfield S, Harris CC. Functional interaction of p53 and BLM DNA helicase in apoptosis. J. Biol. Chem. 276(35):32948-32955, 2001.

Opavsky R, Haviernik P, Jurkovicova D, Garin MT, Copeland NG, Gilbert DJ, Jenkins NA, Bies J, Garfield S, Pastorekova S, Que A, Wolff L. Molecular characterization of the mouse Tem1/endosialin gene regulated by cell density in vitro and expressed in normal tissues in vivo. J. Biol. Chem. 276(42):38795-38807, 2001.

CCR Fluorescence Imaging Facility

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Expert application of fluorescence light microscopy

Mission: The Facility provides support for fluorescence microscopy techniques for laboratories in the Center for Cancer Research. The Facility provides expert scientific consultations on the advantages and limitations of the different forms of microscopy and helps develop a comprehensive microscopy research strategy. The Facility assists in the design of experiments, and helps to solve specific experimental problems associated with the microscopy. The Facility also provides training on the microscopes of choice and technical help during usage of the instruments. The staff of the Facility constantly updates software and hardware and develops new microscopy techniques and experimental approaches. This Facility is oriented to users who want to get first-hand experience in modern microscopy and to carry out their research with state-of-the-art instrumentation and consultation.

Expertise: The NCI Fluorescence Imaging Facility provides equipment and expertise for multi-color high-resolution 2D, 3D and 4D image acquisition on confocal and wide-field microscopes.

Special expertise is provided on FRAP (fluorescence recovery after photobleaching), live imaging and image processing by deconvolution. Acquired images may be processed by several deconvolution packages, including SoftWoRx and XCOSM. Several programs, including Metamorph, Leica confocal and Zeiss confocal, are available for quantification of fluorescence, colocalization studies and texture analysis.

The data are stored on a Terabyte Server and accessible for all users from their personal computers. Image Access database is available for management of the data.

The staff of the facility are experts in fluorescence light microscopy, particularly deconvolution, live imaging, GFP tagging and immunostaining and quantitative image analysis.

Established Technologies:

Multi-color acquisition with optical sectioning for fixed, living cells and tissue sections (up to 7 colors in fixed specimens, GFP, YFP, CFP and DsRed). Confocal technology and image processing by deconvolution may be applied for obtaining high-quality images. Confocal imaging optically reduces out of focus light, thereby improving image contrast. Deconvolution is a post-acquisition computational method used to reduce out-of-focus fluorescence in 3D microscope images.

Access

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Colocalization of proteins by confocal and wide-field microscopy. Software is available for quantification of colocalization.

FRAP. FRAP is a technique to measure the binding and diffusion rates of proteins.

Time-lapse imaging of living cells.

3D reconstructions of cells, basic volume rendering and distance measure between cell structures and chromosomes.

Quantification of protein content and distribution by measuring fluorescence intensity.

New Technologies Under Development:

FRET (Fluorescence Resonance Energy Transfer). FRET is a technique to detect protein interaction and formation of complexes between different proteins tagged with fluorescence markers.

Real-time imaging of living cells. Instrumentation is being tested for rapid data acquisition (up to 30 images per second) at low light levels.

Extended Depth of Focus Microscopy. A new technique is being evaluated for producing a 3D projected image by acquisition of a single focal plane. This technique will have application to rapid live cell imaging; for example, tracking objects that move rapidly in 3D.

Fluorescence Correlation Spectroscopy. This is an approach being tested for measurement of diffusion and binding constants at precise locations within a cell.

Image Access, a database software specialized for managing images. This software is currently being tested by the staff before releasing it to the users of the Facility.

Collaborations:

Dr. Julio Cabrera, NCI, and Dr. Ding Jin, LMB, CCR, NCI. Distribution of RNA polymerase along the *E. coli* chromosome as a function of growth rate and nutrients.

Marta Catalfamo and Dr. Pierre Henkart, CCR, EIB, NCI. Colocalization of endosome proteins.

Ivana Munitic and Dr. Jon Ashwell, LICB, DCT, NCI. The role of T-cell receptor subunit on internalization of the T cell receptor.

Cem Elbi and Dr. Gordon Hager, LRBGE, CCR, NCI. Intracellular distribution of the AhR and ARNT, FRET.

Robert Harrod, BRL; and Dr. Genoveffa Franchini, BRL, DCT, NCI. The effects of HTLV-1 p30 factor on cyclin complexes and protein phosphatases 2A and 1 localization.

Dr. Liusheng He, DCS, NCI; and Amrie Grammer, Autoimmunity Branch, NIAMS. Interactions of signal transduction factors TRAFs with CD40 receptor in human B cells by FRET.

Doc Park and Kyung Lee, LM. Centrosome associated proteins in yeast.

Dr. Marjorie Robert-Guroff, BRL, NCI. Development of RNA FISH assay for detection of HIV infection.

Dr. Shyh-Han Tan and Dr. Mary Dasso, CBMB, NICHD. Role of p21 homolog in the formation of oogenetic syncytium in *Drosophila*.

Luis Parada and Tom Misteli, LRBGE, NCI. The effect of chromosomal localization on translocations.

Dr. Jens Würthner and Dr. Anita Roberts, LCRC. Triple-label imaging of DNA, TGF-b receptor and SMADS.

J. Zhu, S. Petersen, T. Karpova, J.G. McNally, and A. Nussenzweig. (2001) Acute elevation in NBS leads to spontaneous DNA damage, centrosome amplification and death (in preparation).

Publications:

(note that Chris Baumann was Tatiana Karpova's predecessor; *indicates co first authors)

*McNally JG, *Mueller WG, Walker D, Wolford R, Hager GL. The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. Science 287:1262-1265, 2000.

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intranuclear redistribution of peroxisomal proliferator activated receptor isoforms by RXRα. Mol. Endo, submitted, 2001.

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Misteli T, Gunjan A, Hock R, Bustin M, Brown D. Dynamic binding of linker histone H1 to chromatin in living cells. Nature. 408:877-881, 2000.

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NCI Core Genotyping Facility (CGF)

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Division of Cancer Epidemiology and Genetics (DCEG)

HT genotyping, HT sequencing, whole genome scans with microarray technology

Mission: The NCI Core Genotyping Facility was reorganized in October 2001 to provide Genomic support to NCI investigators and programs in the area of high-throughput genotyping and to explore and develop Genomics technology.

Expertise: The NCI Core Genotyping Facility (NCI-CGF) is committed to assisting NCI investigators with technical advice, consultation and high-throughput services related to genotyping. Services available for production are the following: HT sequencing, HT genotyping, Whole Genome scans using Affymetrix HuSNP and fluorescent mapping linkage technology. The center currently provides HT genotyping services using MALDI-TOF mass spectrometry and fluorescent methods, including single-base extension and real-time PCR analysis. The center has extended its ability to process large amounts of DNA samples with a very wide range of quality and concentration, and is extending flexibility to receive DNA samples in various formats.

Established Technologies:

High-throughput sample evaluation/quality control

- Sample arraying/re-arraying
- Fluorescent DNA quantitation
- DNA sample quality evaluation
- DNA sample profiling/fingerprinting/identification

Genotyping:

- MALDI-TOF SNP assay design, production and analysis
- Sequence Detection (Taqman) assay design, production and analysis
- Fluorescent assay design, production and analysis (SNaPshot, VNTR)
- Sequencing (SNP discovery, production and analysis)

Whole genome genotyping/mapping

- Linkage mapping (10 cM and 5 cM resolution)
- DNA Chip analysis (HuSNP)

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Lab/Program Dedicated

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New Technologies Under Development:

NCI-CGF will soon initiate collaborations with major biotechnology companies to help improve systems, software, chemistries, and protocols related to high-throughput genotyping.

Major Equipment at NCI-CGE includes:

1 Sequenom MALDI-TOF Mass Spectrometer

3 Sequenom NanoPlotter robotic nanoliter liquid handling workstations

2 Sequenom/Beckman SpectroPrep liquid handling systems

7 3700-ABI 96-capillary sequencers

3 3100-ABI 16-capillary sequence analyzers

1 Taqman HT7900 sequence detection system

1 Q-8000 BioRobot Qiagen DNA extraction automated system

3 Beckman Multimek 96-channel robotic liquid handling workstation

1 Packard Multiprobe IIex 4 channel robotic liquid handling workstation

1 Beckman Biomek 8-channel robotic liquid handling workstation

3 MJ Research PTC225 Tetrad 384-plate thermal cyclers

4 MJ Research PTC225 Tetrad 96-plate thermal cyclers

8 MJ Research PTC200 96-plate thermal cyclers

2 Affymetrix fluidics workstations

1 Affymetrix hybridization oven

1 Affymetrix-Hewlett Packard GeneArray Scanner

1 Bio-Rad CHEF Mapper Electrophoresis system

1 Bio-Rad Gene Pulser Electroporation system

1 Spectra MAx Plus microtiter plate spectrophotometer

1 Spectra Max Gemini microtiter plate fluorimeter

3 Beckman Allegra 6R Centrifuges

1 Beckman Allegra 21R Centrifuge

2 Beckman Avanti J21 Centrifuges

1 Beckman TLX UltraCentrifuge

4 Savant Speed Vac systems

2 Eppendorf Microfuges

5 Sanyo -80 Ultra-low temperature freezers

4 Sanyo -80 medical freezers

3 Sanyo Medical refrigerators

1 Sanyo Incubator

1 LabLine Laboratory Oven

1 Kodak 120 Gel documentation system

1 Forma Scientific Temperature-controlled orbital shakerreceive DNA samples in various formats.

DNA Sequencing MiniCore Facility

Laboratory of Experimental Carcinogenesis (LEC)

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Sample processing and sequencing and data analysis assistance

Mission: The DNA Sequencing MiniCore Facility accepts samples for DNA sequencing, or microsatellite analysis, from any investigator in the Center for Cancer Research. The facility analyzes these samples using state-of-the-art fluorescence-based sequencers. The MiniCore provides this service for the small-to-intermediate volume users. Most users submit 10 to 20 samples at a time. Emphasis is on analyzing samples rapidly and accurately. Most users receive data within one business day of submission. We also aid investigators in experimental design, interpretation of data, and trouble-shooting.

Expertise: Samples are accepted either for sequencing or for microsatellite analysis. The MiniCore's goal is to generate data for the users as accurately, rapidly, and efficiently as possible. We accept samples where the sequencing reaction has been done by the investigator or we perform the reactions within the core. The samples are electrophoresed on one of four ABI DNA sequencers.

In addition to processing samples for sequencing, we support Macintosh- and PC-compatible software for analyzing the data. We also supply reagents for sequencing. Finally, we frequently help users in experimental design, interpreting their data and in trouble-shooting. Where appropriate, we will collaborate with users in their projects.

Established Technologies:

High-throughput DNA sequencing

DNA sequencing reactions.

Collaborations:

Dr. K.H. Kraemer, BRL, NCI. Analysis of *Xeroderma pigmentosum* families in Turkey and Italy.

B.A. Mock, LG, NCI. Analysis of mouse peroxisomal proteins.

Publications:

Bliskovski V, et al. Structure and localization of mouse *Pmscl1* and *Pmscl2* genes. Genomics. 64(1):106-110, 2000.

Bliskovski V, et al. Mouse peroxisomal protein, Pex14, cDNA sequence, gene structure, and Chr 4 localization. Genomics (in press).

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Gozukara EM, et al. A stop codon in Xeroderma pigmentosum group C families in Turkey and Italy: molecular genetic evidence for a common ancestor. Dermatology (in press).

Laboratory of Molecular Technology
(LMT)

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High-throughput, cutting-edge gene discovery and analysis

Mission: The Laboratory of Molecular Technology (LMT) is an integrated molecular biology laboratory focusing on high-throughput gene discovery and analysis, including advanced sequencing, genetics, genomics, and proteomics technologies together with associated bioinformatics and information management. The LMT is focused on genomic proteomics technology development (including software development) and the application of these technologies to a variety of independent research programs. General access to these advanced technologies is provided to the NCI community through the LMT core service laboratories as described below.

LMT Sequencing Laboratory

LMT Microarray Laboratory

LMT Molecular Diagnostics Laboratory

LMT Oligonucleotide Synthesis Laboratory

Expertise: All of the LMT efforts (including core services, technology development, and independent research) are centered around three core technologies fundamental to modern genomics/proteomics sequencing, genotyping, and microarray. These technologies are in turn supported by robotic and bioinformatic/information management systems, including data analysis tools, database search/comparison tools, and laboratory information management systems (LIMS: sample tracking, data storage and retrieval). Current and developing expertise at the LMT can be summarized as follows:

Oligosynthesis

Specialized oligo production

High-throughput oligo production

Highly purified oligo production

Diagnostics

General DNA diagnostics – research and clinical (CLIA-certified)

Mutation detection

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Sequencing

"Regular" sequencing

High-throughput sequencing

BAC sequencing

Bi-sulfite sequencing (methylation)

Sequence data analysis

Microarray

cDNA microarray production

cDNA microarray analysis

Protein microarray production

Microarray data analysis

Genotyping/mutation detection

SSR genotyping (ABI 377 or 3700)

SNP genotyping (ABI 3700)

SNP genotyping electronic microarrays

Mutation detection (sequencing)

Mutation detection (electronic microarrays)

Laboratory automation

Automated library generation and arraying

Colony picking

General liquid handling/arraying

Automated PCR/sequencing

Automated DNA purification

Automated protein purification

Automated antibody purification

High-throughput monoclonal antibody production

Established Technologies:

High-throughput sequencing

BAC sequencing

Bi-sulfite sequencing (methylation analysis)

Laboratory automation

DNA diagnostics (clinical and research)

Specialized oligonucleotide synthesis

Genotyping (SSR)

Genotyping (SNP)

ABI 3700-mediated genotyping

ABI 377-mediated genotyping

Electronic microarray-mediated genotyping

cDNA microarray production

cDNA microarray analysis

Mutation detection (direct sequencing)

Mutation detection (electronic microarray)

Arrayed library production

High-throughput DNA purification

High-throughput antibody purification

Sequencing data analysis

Microarray data analysis

2-D PAGE data analysis

New Technologies Under Development:

Microarray-based mutation scanning

Oligonucleotide microarrays

Peptide microarrays

Monoclonal antibody microarrays

Sequencing on solid supports

Collaborations:

Dr. John Weinstein, NCI. Methylation.

Dr. Andrew Feinberg, Johns Hopkins University. Methylation.

Dr. Andrew Quest, University of Chile. Caveolin-1 gene function.

Dr. Ping Wang, University of Lund. Signal transduction.

Dr. Walter Rayford, Louisiana State University. Prostate cancer prognostics.

Dr. Kurt Stromberg, FDA. Ovarian cancer metastatic potential.

Dr. H. Rick Bedigian, SAIC, NCI-Frederick. Monoclonal antibody microarrays.

Dr. H. Rick Bedigian, SAIC, NCI-Frederick. Murine ovarian develop ment.

Dr. John Sharp, SAIC, NCI-Frederick. Murine ovarian development.

Nanogen Corporation. Electronic microarray application development.

Publications:

Rasmussen L, et al. A multicenter evaluation of assays for detection of SV40 DNA and results in masked mesothelioma specimens. Cancer Epidemiol. Biomarkers Prev. 10:523-532, 2001.

Dong G, Loukinova E, Chen Z, Gangi L, Chanturita TI, Liu ET, Van Waes C. Molecular profiling of transformed and metastatic murine squamous carcinoma cells by differential display and cDNA microarray reveals altered expression of multiple genes related to growth apoptosis, angiogenesis, and the NK-κB signal pathway. Cancer Res. 61(12):4797-4808, 2001.

NCI Microarray Facility

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cDNA microarrays for gene expression analysis

Mission: Microarray technology is an emerging technology that allows researchers to screen expression levels of thousands of gene sequences, simultaneously generating highly informative molecular profiles for all tissues and cell types. The purpose of the NCI Microarray Facility is to manufacture high quality microarrays for comprehensive gene expression analysis; to provide training, protocols, and technical support to NCI investigators; and to develop new microarray technologies and applications.

Expertise: The NCI Microarray Facility manufactures cDNA microarrays for gene expression analysis and provides both microarrays and technical training to intramural NCI investigators. Our expertise lies in the design, manufacture, quality assurance, and use of microarrays. To design and manufacture arrays, we access cDNAs (gene sequences) via commercial and NCI-related sources as bacterial clones. In 96-well format, plasmid DNA is extracted from the bacterial stocks and used to template high-throughput PCR reactions. Ampified cDNA is purified, quality assessed by fluorometry, robotically aliquoted into 384-well plates, and robotically printed onto lysine-coated glass slides (also prepared in-house). Printed arrays are post-processed for removal of post-printing DNA binding sites on the glass slides and performance-tested prior to distribution. For testing and experimenting purposes, we routinely purify and amplify large amounts of cell line RNA and maintain various RNA species as lab stocks.

Currently, we manufacture and distribute arrays of human, mouse, and rat origin. Various specialized arrays are also manufactured for certain collaborative projects and consortia needs. We also participate in the educational aspects of array use. Each month we host a 2-day training class – free of charge, but mandatory for first-time NCI users. During the first day, participants come to our lab with their own RNA samples and supplies, and together we label cDNAs and set up overnight hybridizations. On the second day we wash, scan, and analyze the arrays, ensuring that the participants are trained to effectively operate the microarray scanner and correctly use the analysis software for calculating expression ratios. Once trained to generate microarray data, array users are then invited to attend an informatics training class hosted by our partners in the CIT Section of Bioinformatics and Molecular Analysis. This class is designed to teach investigators how to upload their array data to the NCI-CIT database (mAdb), assemble their data into project folders, and utilize analytical tools for multi-array analysis.

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We are also committed to technology development. To this end we have expanded the capabilities of array technology in the analysis of small amounts of RNA, otherwise limiting amounts of RNA, such as those derived from core biopsies and fine needle aspirates. In a recent publication, we describe our method of RNA amplification which yields tens of micrograms of amplified mRNA from tens of nanograms of total RNA, and we validate the use of this approach in gene expression studies (E Wang, LD Miller, GA Ohnmacht, ET Liu, FM Marincola. 2000. High-fidelity mRNA amplification for gene expression profiling. Nature Biotechnology 18(4): 457-459). We are also committed to developing new technical approaches and streamlining existing protocols in order to increase efficiency and quality of array manufacture and use. Technical advances we have implemented have both greatly increased our output potential as well as reduced overhead costs associated with array manufacture.

Established Technologies: We provide

Expression arrays of human, mouse, and rat origin

Training programs for array hybridization and analysis

Online protocols and information updates via e-mail.

New Technologies Under Development:

Oligo-based microarrays composed of 60-mer oligos BLAST-searched against existing EST and genomic databases for low cross-homology with other sequences

New printing strategies/technologies for higher speed, density, and quality printing

Inexpensive "TestHyb" arrays for probe quality evaluation prior to hybridization with more expensive arrays

"Transcriptome" arrays for assessing expression patterns of specific genes in the context of different cell lines, tissues, and states of development, differentiation, and disease.

Collaborations:

Dr. Franco Marincola, NCI. RNA amplification gene expression analysis; gene profiling using transcriptome-based arrays.

Dr. Sheue-Yann Cheng, NCI. Identification of thyroid hormone-regulated genes and thyroid hormone-mediated repression of the Wnt signaling pathway; multi-tissue expression profiling in mouse models of human thyroid hormone resistance.

Dr. Silvio Gutkind, NIDCR. Dissecting transcriptional programs in response to chemotherapuetic agents.

Dr. Jim Mitchell, NCI. Studying the effects of radiation on different tissues in vitro and in vivo.

Dr. Jeff Miller, NIDDK. The Echip: designing a microarray array for gene expression analysis in differentiating erythrocytes.

Howard Fine, NCI. Creating a specialized array for brain tumor studies.

Dr. Michael Birrer, NCI. Creating a specialized array for ovarian tumor studies.

Compugen (pre-CRADA). Performance testing of oligo-based microarrays and comparison to cDNA microarray performance.

MMHCC consortium. Providing arrays and technical and analytical support for four research labs studying mouse models of human cancer:

Charles Sawyers (UCLA)

Robert Matusik (Vanderbilt)

David Largaespada (University of Minnesota)

Terry VanDyke (UNC Chapel Hill)

Publications:

Wang E, Miller LD, Ohnmacht GA, Liu ET, Marincola FM. High-fidelity mRNA amplification for gene profiling. Nat. Biotechnol. Apr;18(4):457-459, 2000.

Gene Expression Laboratory
(GEL)

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Real-time PCR quantification of nucleic acids, recombinant adenoviruses, bulk RNA preparation from cells and tissues

Mission: The Gene Expression Laboratory provides scientific expertise in mRNA isolation and purification from a variety of biological specimens and characterization and quantification of nucleic acids, including real-time PCR technology. It also provides scientific and technical expertise necessary to generate and produce recombinant adenoviruses for gene expression studies.

Expertise: The Real-Time PCR Core Facility provides expertise in designing assays to quantify the relative or absolute levels of specific genes, at both DNA and RNA levels over a broad dynamic range. Expertise also includes total or cytoplasmic RNA and poly A RNA preparation, cDNA synthesis, RT-PCR.

The Adenovirus Production Group provides expertise in generating and characterizing recombinant adenoviruses, amplification and purification of high titer viruses in bulk quantities.

The Support to Mammalian Gene Collection Group provides support services to the NCI Director's Challenge projects (CGAP, MGC, Lympho-Pool and Analytical Tool Projects) in the area of establishing a biological model system to enrich certain classes of mRNA and their isolation, purification and quality control analysis (from cells and tissues).

Established Technologies:

Real-Time PCR Technology Group

- Real-Time PCR assay design and development
- Establishment of dual labeled probes
- Establishment of endogenous controls
- Total or cytoplasmic RNA and poly A RNA preparation
- cDNA synthesis
- Set up and run assay
- Data analyses

Adenovirus Group

- Adenovirus production and purification

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Adenovirus expression vector engineering

Recombinant adenovirus generation

Crude viral lysate preparation

Virus amplification and purification

Titer determination

Support to Mammalian Gene Collection Program

Establishment of RNA isolation and purification protocols

Production of large quantity of cells representing different human tissue types

Cytoplasmic poly A RNA preparation

Quality control analysis

Establishment of a biological model system to enrich certain classes of mRNA during cell proliferation, differentiation and cell death.

Collaborations:

Dr. Libutti, Dr. Harris, NCI, NIH. Recombinant adenovirus generation starting from expression vectorDNA.

Dr. Strausberg, NCI, NIH. Mammalian gene collection program: Establishing biological model system (T-cell activation, blood cell differentiation) to enrich certain classes of mRNA.

Publications:

Romano-Spica V, Mucci N, Ursini CL, Ianni A, Bhat NK. ETS1 induction by ELF-modulated radio frequency electromagnetic field. Bioelectromagnetics. 21:8-18, 2000.

Woodson K, Stewart C, Barret M, Bhat NK, Viratamo J, Taylor PR, Albanes D. Effect of vitamin intervention on the relationship between GSTM1, smoking, and lung cancer risk among male smokers. Cancer Epidemiol. Biomarkers Prev. 11:965-970, 1999.

Woodson K, Ratnasinghe D, Bhat NK, Stewart C, Tangrea JA, Hartman TJ, Stolzenberg-Solomon R, Virtamo J, Taylor PR, Albanes D. Prevalence of disease-related DNA polymorphisms among participants in a large cancer prevention trial. Eur. J. Cancer Prev. 8:441-447, 1999.

Protein Chemistry Core

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Analysis of dynamic changes in proteins

Mission: The Protein Chemistry Core provides NCI with expertise and experience in protein chemistry with an emphasis on the dynamic characterization of protein phosphorylation.

Expertise: The Core maps phosphorylation sites in proteins using ^{32}P techniques. This approach provides information on the dynamic nature rather than the resting state of protein phosphorylation. Capillary electrophoresis is used to measure the change in mass/charge ratio when a ligand binds to a protein or peptide providing binding constants. Amino acid analysis provides the composition and quantitation of peptides and proteins.

Peptide mapping by HPLC and 2D electrophoresis

Kinetic analysis of proteases with peptide substrates and HPLC

Molecular weight determination of peptides and proteins by electrospray mass spectrometry.

Established Technologies:

Protein and peptide N-terminal sequencing

Amino acid analysis – composition and quantitation

HPLC peptide mapping

2D peptide and protein mapping

^{32}P phospho-amino acid analysis

Mapping phosphorylation sites using ^{32}P

Capillary electrophoresis

Kinetic analysis of proteases

ELISA assays

Molecular weight determination of peptides and proteins by electrospray mass spectrometry.

New Technologies Under Development:

Use of capillary electrophoresis to measure the binding of ligands to proteins and peptides

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Increased sensitivity of mapping phosphorylation sites

^{32}P 2D maps to image the dynamic nature of phosphorylation in proteins.

Collaborations:

Dr. Deborah Morrison, CCR, NCI-Frederick. Mapping dynamic status of phosphorylation in proteins.

Dr. Peter Johnson, CCR, NCI-Frederick. Mapping phosphorylation sites in proteins.

Dr. Karen Vousden, CCR, NCI-Frederick. Mapping phosphorylation sites in a protein.

Dr. Stephen Shaw, CCR, NCI. Mapping autophosphorylation sites in a protein.

Dr. Kazimierz Kasprzak, NCI-Frederick. Molecular weight determination.

Dr. David Waugh, CCR, NCI-Frederick. Protease kinetics.

Publications:

Tozser J, Zahuczky G, Bagossi P, Louis JM, Copeland TD, Oroszlan S, Harrison RW, Weber IT. Comparison of the substrate specificity of the human T-cell leukemia virus and human immunodeficiency virus proteinases. Eur. J. Biochem. 267:6287-6295, 2000.

Phylip LH, Lees WE, Brownsey BG, Bur D, Dunn BM, Winther JR, Gustchina A, Li M, Copeland T, Wlodawer A, Kay J. The potency and specificity of the interaction between the IA3 inhibitor and its target aspartic proteinase from *Saccharomyces cerevisiae*. J. Biol. Chem. 276:2023-2030, 2001.

Minakuchi M, Kakazu N, Gorrin-Rivas MJ, Abe T, Copeland TD, Ueda K, Adachi Y. Identification and characterization of SEB, a novel protein that binds to the acute undifferentiated leukemia-associated protein SET. Eur. J. Biochem. 268:1340-1351, 2001.

Evdokimov AG, Tropea JE, Routzahn KM, Copeland TD, Waugh DS. Structure of the N-terminal domain of *Yersinia pestis* YopH at 2.0 Å resolution. Acta Crystallogr. D57:793-799, 2001.

Sharp AH, Black JL, Dubel SJ, Sundarraj S, Shen J, Yunker AM, Copeland TD, McEnery MW. Biochemical and anatomical evidence for specialized voltage-dependent calcium channel gamma isoform expression in the epileptic and ataxic mouse, stargazer. Neuroscience, 105:599-617, 2001.

Muller J, Ory S, Copeland T, Piwnica-Worms H, Morrison DK. C-TAK1 regulates Ras signaling by phosphorylating the MAPK scaffold, KSR1. Mol. Cell. 8:983-993, 2001.

Miura K, Miyazawa S, Furuta S, Mitsushita J, Kamijo K, Ishida H, Miki T, Suzukawa K, Resau J, Copeland TD, Kamata T. The Sos1-Rac1 signaling: possible involvement of a vacuolar H^+-ATPase E subunit. J. Biol. Chem. 276:46276-46283, 2001.

Protein Chemistry Laboratory
(PCL)

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Protein, peptide, and oligonucleotide analysis, purification and characterization

Mission: The Protein Chemistry Laboratory (PCL) provides high sensitivity (picomole to femtomole) protein identification using methods that are classic (Edman sequencing) to methods that are relatively new (mass spectrometry). We are expert at macromolecular interactions employing surface plasmon resonance spectroscopy and then using this information as a basis for a molecular mechanism-based macromolecular screen for small molecule inhibitors.

Expertise: The PCL performs high-sensitivity, state-of-the-art, N-terminal amino acid sequencing, and provides expertise in:

- Protein, peptide, and oligonucleotide analysis and purification by high-performance liquid chromatography (HPLC)
- Protein identification and characterization of post-translational modifications by mass spectrometric techniques
- Identification of proteins and peptides by microsequencing using mass spectrometry and Edman degradation
- Surface plasmon resonance (SPR) analysis of molecular interactions
- Molecular mechanism-based small molecule inhibitor screening.

High-Performance Liquid Chromatography. We perform, in-house, the final purification and analysis of all proteins, peptides, and oligonucleotides that are used in projects conducted by our laboratory.

Protein Identification and Characterization of Post-Translational Modifications by Mass Spectrometric Techniques. Using the Voyager Pro DE MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometer, we have identified proteins using gel digestion with trypsin or via a chemical cleavage method developed by our laboratory. These methods have helped us both to identify and delineate the post-translational modifications of proteins.

Identification of Proteins by Microsequencing of Proteins and Peptides Using Edman Degradation. The PCL's ABI Procise (model 494) sequencer amino acids microsequencer equipped with a capillary HPLC phenylthiohydantoin (PTH) amino acid analyzer provides routine amino acid sequencing in the low picomole to high femtomole range.

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Surface Plasmon Resonance (SPR) Spectroscopy. SPR spectroscopy analysis provides an investigator with in-depth data related to the biophysical and biochemical behavior of a target macromolecule(s) and its interactions with specific cognate molecules. We use the BIAcore® instrument to acquire real-time signals that detail the molecular interactions between a tethered ligand and solution analytes. The resulting signals are rich in interaction information. The PCL's scientific staff collaborates with the Statistical Services Group (Data Management Services, Inc., NCI-Frederick) to model and extract the information contained in these signals. This collaboration, although mathematically demanding, is producing new insights into macromolecule interactions that are expected to be useful in experimental design and in characterizing ligand binding.

Molecular Mechanism-Based Small Molecule Inhibitor Screen. The positive results that we obtained through SPR analysis on several studies suggested to us that these interactions could provide a useful basis for finding small molecules that interfere with a specific macromolecular function.

Established Technologies:

Molecular Interactions Group (MIG)

Biomolecular interaction analysis (BIA) via BIAcore® technology requires four main steps for experimental set-up:

Immobilization: Linking of one of the interacting molecules to the sensor chip surface. Different linking chemistries and conditions may have to be explored to optimize this step.

Binding: Injection of the interaction partner across the immobilized ligand surface and signal production. Appropriate concentrations for the interaction will be investigated and determined.

Regeneration: The process of removing the bound analyte from the ligand surface. Regeneration allows for re-use of the surface for determination of concentration effects of ligand-analyte interaction.

Controls: Appropriate controls must be designed to verify that the observed binding is specific and that non-specific binding is eliminated.

Upon the successful completion of these steps, data analysis allows for the determination of various kinetic parameters for the binding partners, including association and dissociation rates.

Molecular Mechanism-Based Screening Group

Determine optimal binding conditions of reactants using surface plasmon resonance (BIAcore® technology). This allows real-time binding information for the reaction, which is important in optimizing the assay.

Translate the optimized assay into a 96-well plate format. This step will also require additional optimization for parameters such as reactant concentrations, reaction volume, plate type and the stability of the assay (time dependence). A bioluminescence detection system is routinely used as this is highly sensitive, stable and is not affected by colored or fluorescent compounds.

Automation of the screen. Once the 96-well plate assay is optimized, the assay is made compatible with a robotic workstation (Tomtec Quadra). Each plate has 8 positive and 8 negative controls, and statistical analysis of the dynamic range and data variation is determined for each plate.

The screen is then performed with the Diversity Set of 2000 compounds that represent all the different chemotypes found in the NCI's repository of 140,000 chemical compounds.

Hit compounds are then validated using BIAcore® technology. A generalized hit structure is used to search the NCI repository for related compounds. Binding constants are measured for each compound using BIAcore® technology or other biochemical methods (tryptophan fluorescence quenching, fluorescence polarization).

Structure-activity relationships. Analysis of the binding constants for related compounds can provide information on the chemical groups that are important for binding.

Protein Identification Group

Chemical amino acid sequencing of low picomole to high femtomole amounts of protein electroblotted onto PVDF membranes.

Enzymatic digestion of proteins, separation by HPLC and identification by MALDI-TOF Mass Spectrometry or Edman amino acid sequencing.

In-gel in-solution digestion, zip-tip desalting or enrichment, peptide mass mapping.

New Technologies Under Development: Method Development for Protein Identification

Protein identification by peptide mass mapping has become a routine. However, improvement in cleavage efficiency, specificity is key to the successful identification/detection of membrane proteins and minor proteins. We have developed chemical cleavage by mild acid hydrolysis as an alternative to enzymatic digestion. This method needs to be fine-tuned and further tested. In addition, we need to establish protocols for quantification, enrichment and detection of phosphopeptides to enhance the protein identification function of the Protein Chemistry Laboratory.

Molecular Mechanism-based Screens: Finding the Needle in the Haystack.

In a recent molecular mechanism-based screen, designed to identify high-affinity, non-covalent reversible inhibitors of HIV nucleocapsid p7 protein (NC-p7), a series of compounds were identified as positives. These positives were confirmed and binding constants calculated using both Surface Plasmon Resonance Spectroscopy (BIAcore® analysis) and tryptophan fluorescence. Unfortunately, further analysis of these positive compounds has demonstrated that in the majority of cases they are not homogeneous, and in fact, the predicted compound is not even present in the mixture. Many of the chemicals in the NCI's repository could have been impure when they were donated or could have deteriorated over time. Currently work is underway to purify the active component in many of these mixtures. In conjunction with this effort it is proposed that a micro-purification scheme be developed such that compounds that bind NC-p7 immobilized on a 96-well plate could be identified by mass spectrometry. Methods will be developed to elute the NC-p7 “captured” small molecule from the plate in a buffer compatible for mass analysis. Electrospray mass spectrometry will be used to determine the mass of the small molecules (a new QTOF with multi-ionization source will be available towards the end of the summer). This kind of micro-purification scheme could be adapted for use with other high-throughput assays in the future.

Molecular Interactions Group (MIG)

MIG Research Components:

The Molecular Interactions Group (MIG) in the Protein Chemistry Laboratory, SAIC-Frederick, Inc., has as its primary function, the study of biomolecular interactions, including protein-protein, protein-oligonucleotide and biopolymer-ligand binding. Central to these studies is the use of BIAcore® technology for the interrogation of these interactions. Currently there are two projects that constitute research initiatives within MIG. The first is the ongoing investigation of HIV nucleocapsid p7 protein (NC-p7) and its interaction with oligonucleotides. This work has been of interest in the laboratory for several years and is cumulating currently with a proposal for a novel mechanism of binding which includes a ternary interaction of p7 and oligo. The understanding of the binding mechanism is of importance not only for theoretical implications, but is also directly related to molecular target studies of potential small molecule drug candidates which can disrupt this binding, and therefore, be of value in the treatment of AIDS.

A second area of research is the coupling of BIAcore® with mass spectrometry and, in a more general approach, affinity capture with mass spectrometry. The coupling of these technologies will provide a route to low-level, complete characterization of protein interactions. One disadvantage of the detection system used in BIAcore® (surface plasmon resonance) is that it is an indirect measurement of the interaction of the bound surface material and the ligand. The coupling of mass spectrometry will allow for direct mass measurement of the components in the binding study. In addition to BIAcore® chips, other affinity capture techniques will be investigated for purification and pre-concentration prior to mass spectrometric analysis.

Collaborations:

Dr. Steven Shaw, CCR, NCI. Mapping phosphorylation sites on protein kinase theta.

Dr. David Garfinkle, CCR, NCI-Frederick. Protein identification using mild acid cleavage and MALDI-TOF mass spectrometry.

Dr. Larry Keefer, CCR, NCI-Frederick. Mapping nitrosylated amino acids by mass spectrometry.

Dr. Alan Rein, DRP, CCR, NCI-Frederick. HIV NCp7 interactions with nucleic acids.

Luke Ratnasinghe, CCR, NCI. Identification of biomarkers for cancer in serum.

Dr. Robert Shoemaker, DTP, NCI. Molecular mechanism-based screen for small molecule inhibitors.

Jonathan Vogel, Dermatology Branch, NCI. Analysis of membrane proteins.

Publications:

Medaglia MV, Fisher RJ. Analysis of interacting proteins with surface plasmon resonance spectroscopy using BIAcore® Protocol 6. In *Molecular Cloning: A Laboratory Manual*, Third Edition. J. Sambrook, P. MacCallum, D. Russell, eds. Cold Spring Harbor, NY: Cold Spring Harbor Press, 2001. p. 18.99-18.117.

Padow M, Lai L, Fisher RJ, Zhou YV, Wu X, Kappes JC, Towler EM. Analysis of Human Immunodeficiency Virus Type 1 containing HERV-K protease. AIDS Res. Hum. Retroviruses. 16(18):1973-1980, 2000.

Biophysics Resource in the Structural Biophysics Laboratory (SBL)

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Dedicated support for biophysical analysis

Mission: The Biophysics Resource (BR) in the Structural Biophysics Laboratory provides biophysical instrumentation and needed assistance for users from the Center for Cancer Research, NCI. The BR is a shared use facility and does not provide or perform drop-off service or support. Investigators or their staff may visit the BR facility and use the instrumentation according to the policies indicated at http://sblweb.ncifcrf.gov. The access mode for each instrument is determined by BR personnel.

Expertise: The Biophysics Resource in the Structural Biophysics Laboratory provides cutting-edge biophysics technologies in

CD spectroscopy for studies of optical activity and conformation of biomacromolecules

Steady-state fluorescence spectroscopy for studies of structure and environment of biomacromolecules and mechanism of their interactions

Isothermal titration calorimetry for thermodynamic characterization of biomacromolecular interactions

Liquid chromatography with mass spectrometry detection for macromolecular mass characterization

Multi-angle laser light scattering for determination of macromolecular mass and size.

Established Technologies: The Biophysics Resource in the Structural Biophysics Laboratory provides the following technologies

CD spectroscopy

Steady-state fluorescence spectroscopy

Isothermal titration calorimetry

Liquid chromatography with mass spectrometry detection

Multi-angle laser light scattering (batch mode) for determination of macromolecular mass and size.

New Technologies Under Development: Multi-angle laser light scattering (chromatography mode) for determination of macromolecular mass distribution, macromolecular shape, degree of aggregation.

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Lab/Program Dedicated

Collaborations:

Dr. Chris Michejda, Structural Biophysics Laboratory, CCR, NCI-Frederick. Fluorescence spectroscopy study of drug-DNA interactions.

Dr. David Waugh, Macromolecular Crystallography Laboratory, CCR, NCI-Frederick. IT-calorimetry characterization of maltose-binding protein and oligo saccarides interaction.

Dr. Alexander Wlodawer, Macromolecular Crystallography Laboratory, CCR, NCI-Frederick. IT-calorimetry characterization of phosphodiesterase-substrate interactions.

Publications:

Evdokimov AG, Anderson DE, Routzahn DM, Waugh DS. Structural basis for oligosaccaride recognition by *Pyrococcus furiosus* maltodextrine-binding protein. J. Mol. Biol. 305:891-904, 2001.

Protein Expression Laboratory, (PEL)

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Expression of recombinant proteins

Mission: To facilitate the cloning, expression, and purification of active proteins from eukaryotic and prokaryotic cells.

Expertise:

Cloning into multiple expression systems: We use the Gateway recombinational cloning technology to clone open reading frames into multiple vectors to optimize expression in *E. coli*, insect, and mammalian cells.

Parallel expression validation: We are establishing the capability to examine up to 48 combinations of vector promoter, vector fusion tag, *E. coli* strain, growth temperature, and induction condition, in parallel, so as to maximize gene expression, solubility, and protein activity. We anticipate extending this to insect and mammalian cells.

Parallel tagged protein purification: We expect to be able to purify four or more tagged proteins in parallel, enabling, for example, rapid turnaround of mutants, or assessment of native, amino, or carboxy purification tags.

Microbial cell fermentation (MCF): Shake flasks, 20-liter and 60-liter tanks, *E. coli* to 150 OD's, yeast, SDS-PAGE assay of protein expression.

Eukaryotic cell production (ECP): Culture of many mammalian and insect cell lines, for recombinant protein, antibody, and monoclonal production. ELISA assays.

Protein Purification Lab (PPL): Recombinant proteins from all cell types, native or tagged, up to 100's of mg, using all standard methodologies. Light scattering for assessing monomeric or multimeric status of the purified protein.

Established Technologies:

Vector Engineering: PCR techniques, vector engineering using the Gateway Technology, transformation and transfection of organisms and cells, expression in bacteria, eukaryotic cells and baculovirus systems, analysis of protein production.

Eukaryotic Cell Production: Culturing of many different mammalian cell lines and insect cell lines, production of antibodies and small-scale purification of those antibodies using protein G or A columns, and ELISAs.

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Microbial Cell Fermentation: Shake-flask and 60-liter fermentation. The 60-liter fermentations can be performed in tandem to give 120-liter capacity. Gram-negative, gram-positive, and several different strains of yeast can be fermented.

Protein Purification: Affinity columns, anion exchange columns, cation exchange columns, concentration, light scattering.

Protein Purification Core (PPC)

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Basic Research Laboratory, NCI-Frederick

Expertise in protein purification for the NCI

Mission: The mission of the PPC is to provide research support to the NCI investigator requiring small-scale (1-2 mg) or large-scale (50-100 mg) protein purification.

Expertise: The PPC provides expertise in protein purification to the NCI community. The staff has a wide range of experience with both native and recombinant proteins produced in bacteria, yeast, insect cells and mammalian cells. This experience includes the purification of soluble and membrane-bound proteins, glycoproteins, fusion proteins and tagged proteins. The core can also offer methods development when no defined purification stategy is available.

Established Technologies: The PPC uses the following techniques for the purification and characterization of the target proteins.

- Affinity chromatography
- Ion exchange chromatography
- Hydrophobic interaction chromatography
- Dye chromatography
- Hydroxyapatite chromatography
- Lectin chromatography
- Gel filtration chromatography
- Isoelectric focusing
- Chromatofocusing
- Gel electrophoresis
- Western analysis
- Enzyme analysis
- Immunoprecipitation
- Electrospray mass spectrometry

New Technologies Under Development: None at present.

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Publications:

Evdokimov AG, Tropea JE, Routzahn KM, Copeland TD, Waugh DS. Structure of the N-terminal domain of *Yersinia pestis* YopH at 2.0 Å resolution. Acta Crystallogr. D57:793-799, 2001.

Evdokimov AG, Tropea JE, Routzahn KM, Waugh DS. Structure of the RhoGAP domain of *Yersinia pestis* YopE. Protein Sci. (submitted).

Evdokimov AG, Tropea JE, Routzahn KM, Waugh DS. Structure of the *Yersinia pestis* chaperone protein SycE. Acta Crystallogr. (submitted).

Kapust RB, Tozser J, Fox JD, Anderson DE, Cherry S, Copeland TD, Waugh DS. Tobacco Etch Virus protease: Mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency. Biochemistry (submitted).

Comparative Molecular Cytogenetics Core Facility

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Genetics Branch, CCR, NCI

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Assessment of structural and numerical genomic change in cancer, pre-cancer, and cancer models

Mission: The Genetics Branch of the Center for Cancer Research, NCI, is developing a comparative molecular cytogenetics core facility to provide molecular cytogenetic support services to the NCI community. The comparative molecular cytogenetics core will facilitate the assessment of structural and numerical genomic changes in cancer, pre-cancer, and cancer models. Currently service components of this core facility include fluorescent in situ hybridization (FISH), spectral karyotyping (SKY), comparative genome hybridization (CGH), and chromosome flow sorting. Matrix or array comparative genomic hybridization (aCGH) analyses are envisioned for the near future.

Expertise:

FISH – FISH (Fluorescence in situ hybridization) includes hybridization with a variety of probes of various sizes and complexity. FISH can be done with probes specific to entire chromosomes or of decreasing sizes such as YACs, BACs, PACs and cosmids. Current size limitations of FISH are on the order of 1 to 2 MB. Attention can be focused on specific genomic regions that might be hot spots of cell lineage-specific chromosomal rearrangement and/or selected because of alterations that they cause in gene expression or function.

Specific genes or genomic segments can be hybridized either to metaphases or interphase cells. When hybridized to metaphase cells, probes can be mapped to a specific chromosome region. When hybridized to interphase cells, the copy number of the region in a cell or cell population can be assessed, as can its context vis-à-vis its nearest genomic neighboring segments. Interphase cytogenetics has an advantage in that there is no need for actively dividing cells.

SKY – SKY (Spectral Karyotyping) is a multi-color extension of FISH. With SKY it is possible to simultaneously "paint" each chromosome of a species in its own defining "color". This technique permits the identification of all inter-chromosomal rearrangements (translocations) in a single hybridization experiment. In SKY each chromosome is labeled with a distinctive combination of fluorescent haptens.

After hybridization to target metaphases and detection, imaging is accomplished using an optical microscope equipped with a CCD camera and a spectral cube that can simultaneously measure emission spectra for each pixel. Structural chromosomal rearrangements can be identified (even by

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non-cytogeneticists) without detailed knowledge of chromosome-specific Giemsa (G)-banding patterns. Segments of chromosomes without distinctive G-banded landmarks can still be identified even if embedded in complex or subtle chromosome translocations. This technological advance allows a rapid, refined definition of specific structural genomic changes in cancer or evolution.

CGH – CGH (Comparative Genome Hybridization) has been extensively applied to define chromosome gains and losses and gene amplification in human tumors. The use of CGH was recently extended to the analysis of mouse tumors, thus providing important information on the mechanisms of chromosomal aberrations occurring during carcinogenesis. Genomic tumor and control DNA are labeled with different fluorochromes and hybridized onto normal metaphases in the presence of unlabeled cot-1 DNA. The quantitative analysis of the different fluorescent intensities identifies the chromosomal regions that are recurrently gained and lost in the respective tumors. CGH data can be compared to the results obtained by SKY or FISH.

Chromosome Flow Sorting – Chromosomes can also be analysed by preparing a monodispersed suspension of chromosomes, staining with fluorochromes and passing them through a flow cytometer. The intensity of each chromosome depends on its DNA content and AT/GC ratio. Chromosomes can also be sorted and chromosome paints can be made by degenerate oligonucleotide-primed (DOP) PCR amplification. These paints can then be hybridized to metaphases or combined to establish SKY kits from any desired species. Chromosome flow sorting of tumors allows reverse painting to study complex chromosomal rearrangements.

In reverse painting, probes are generated by sorting the rearranged chromosomes from the patient and then hybridizing to normal metaphase spreads. Insight is acquired about the origin of the marker chromosomes and breakpoints involved. Reverse painting also allows a more precise sub-chromosomal assignment for marker chromosomes when chromosomes have been fragmented by multiple translocations and adds information about breakpoints involved in chromosome evolution during tumorigenesis. Phage and cosmid libraries can also be constructed from flow-sorted chromosomes.

Established Technologies:

Fluorescent in-situ hybridization (FISH)

Spectral Karyotyping (SKY)

Comparative Genome Hybridization (CGH)

Flow sorting of chromosomes from distinct species (human, murine, non-human primates etc.)

New Technologies Under Development:

aCGH – The Genetics Branch has taken the lead in the establishment of a human high resolution FISH-mapped set of bacterial artificial chromosomes (BACs) covering the human genome at 1-2 Mb spacing. This set (the "CCAP" set) of mapped and sequenced clones (the first version of which is expected to be completed by the end of 2001) links the cytogenetic and physical maps of the human genome. The data are accessible on a web site that is also linked to extant maps and cancer cytogenetic databases. The clones themselves are available from Research Genetics. A related effort is now underway to establish an analogous set of murine clones.

An anticipated activity of the core facility will be to print these human and murine clone sets in a microarray format. Such a format will provide a hybridization platform that will allow assessment of regional genome copy number changes (gains and losses) at a 1-2 Mb resolution and in a format that will provide the kind of enhanced analytic software advantages that are currently under development for other microarray applications. The use of such arrays would include definition of genome changes in human cancers, precancers, or murine cancer models, the study of genome change as a biomarker of cancer risk, diagnostic or prognostic assessments, etc. While attention has been focused on the use of BAC clones, it is also possible to array cDNAs in a similar format to assess copy number change. DNA amplification of specific genes can be demonstrated by this process (demonstrating single copy deletion is more problematic for cDNAs at the moment). The hardware/software for making and imaging these arrays is currently under development and is relatively costly.

Advanced Biomedical Computing Center (ABCC)

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A high-performance, scientific computing resource supporting genomic and proteomic studies

Mission: The ABCC is the high-performance computing and computational biology center for the NIH/NCI. The facility is for all intramural investigators of the NIH and NCI. The ABCC has numerous computer platforms representing both fast scalar and vector computing.

Expertise: The ABCC has a wide range of computational tools ranging from bioinformatics to modeling to quantum chemistry.

The ABCC computer staff maintains computational tools, computers, and provides large storage and archival facilities.

The scientific staff provides HPC resources for both NIH/NCI intramural scientists and the extramural biomedical research community. Its mission is to provide HPC support, collaborate in research, and to conduct in-house research in various areas of computational biology and biomedical research.

Established Technologies:

- Bioinformatics
- Computational chemistry
- Networking
- Videoconferencing
- Web services
- E-mail
- Computer network security
- Molecular modeling
- Inhibitor design
- Database screening
- Consultation
- Training

New Technologies Under Development: Use of high-performance computing for genome analysis and protein family prediction.

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Graphic design and visual communications

Mission: Our purpose is to optimize and facilitate the effective communication of NCI-Frederick investigators to the scientific community and the general public. To achieve that mission, the Publications Department delivers a full range of specialized products and services to meet the presentation and publication needs of the scientific community.

Expertise: The staff at Publications possess expertise in graphic design, scientific illustration, photographic and digital imaging, writing, editing, and other preparation of materials for a wide variety of media. With as many as 20 different software programs and varied hardware configurations, the Publications staff creates exceptional products. The department creates sophisticated artwork for products such as slides, site visit presentations, poster sessions, Web sites, journal submissions and reports, as well as enhances or revises many clients' digital artwork for slides and prints. Representative services include:

Design/provide artwork/electronic files for covers of scientific journals and for journal submissions

Design posters and on-screen shows for the presentation of scientific accomplishments at local, national and international meetings

Create figures for patent applications

Design, produce and maintain Web sites

Create modular and one-piece posters, flyers, handouts, signs, award certificates, and other materials for various NCI-Frederick events, recruitment opportunities

Provide photography for site visits, scientific documentation, special events, recruitment posters, as well as in-house photographic production – passport photography, scanning, digital imagery, slide film development and slide duplication

Edit manuscripts, scientific reviews, book chapters, reports, and materials for special events; edit and lay out newsletters; revise manuals; provide daily quality control through editing/proofing slides, figures, charts, structures, prints, poster pieces, flyers, memos, and other materials

Transcribe gene sequences and equations; generate mass mailings; and laminate scientific materials

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Lab/Program Dedicated

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Create and provide business cards, stationery, notepads, complex tables, name tents, laboratory and business forms, cage cards, lab manuals, reports, newsletters, telephone directory and Human Resources materials

Established Technologies:

Photographic imaging and processing

Image processing—scanning, photographic and digital technologies

Word processing and electronic publishing

File transfer

Computer-generated graphics and visuals

Digital-to-film imaging

Digital-to-hard copy imaging

Contract for offset and digital printing

New Technologies Under Development:

Web design: We are designing Web sites for a number of on-site clients, as well as revising the NCI-Frederick Web site to make it more client-friendly.

Further development of existing technology is planned.

Collaborations:

The work we do and the services we provide are by nature collaborative processes. Completing an average of 250 jobs per month, Publications staff spend much time collaborating with clients from the NCI, SAIC-Frederick, Inc., Charles Rivers Laboratories, U.S. Army Medical Research Institute of Infectious Diseases, and Data Management Services. Often, the illustrator, photographer, editor or document processor works with a client to develop the job. The collaboration may take place through several proof cycles, with the client spending a half hour or more at each meeting with the staff to develop the project in a way that meets the scientist's vision and clearly conveys his/her ideas.

Publications:

While Publications staff members did not write any of the following articles, they made significant contributions to these and others during the past year by providing original artwork, photography and other imaging, and editorial expertise.

Bal W, Liang R, Lukszo J, Lee SH, Dizdaroglu M, Kasprzak KS. Nickel(II) specifically cleaves the C-terminal tail of the major variant of histone H2A and forms oxidative damage-mediating complex with the cleaved-off octapeptide. Chem. Res. Toxicol. 13:616-624, 2000. (art work)

Bal W, Dyba M, Szewczuk Z, Jezowska-Bojczuk M, Lukszo J, Ramakrishnana G, Kasprzak KS. Differential binding of Zn(II) and DNA by partial peptides of human protamine HP2. Mol. Cell Biochem. (art work)

Bal W, Wojcik J, Macidjczyk M, Grochowski P, Kasprzak K. Induction of a secondary structure in the N-terminal pentadecapeptide of human protamine HPd2 through Ni(II) coordination. An NMR study. Chem. Res. Toxicol. 13(9):823-830, 2000. (cover art work)

Buzard GS, Kasprzak KS. Possible roles of nitric oxide and redox cell signaling in metal-induced toxicity and carcinogenesis. A review. J. Environ. Pathol. Toxicol. Oncol. 19:179-199, 2000. (art work)

Dai RM, Li C-C. Valosin-containing protein is a multi-ubiquitin chain-targeting factor required in ubiquitin-proteosome degradation. Nat. Cell. Biol. 3(7):740-744, 2001. (art work)

Davies KM, Wink DA, Saavedra JE, Keefer LK. Chemistry of the diazeniumdiolates. 2. Kinetics and mechanism of dissociation to nitric oxide in aqueous solution. J. A. Chem. Soc. 123(23):5473-5481. (art work)

Kasprzak KS, Buzard GS. The role of metals in oxidative damage and redox cell-signaling derangement. In: Molecular Biology and Toxicology of Metals, RK Zalups, J Koropatnick, eds, pp 477-505, Taylor and Francis Inc., New York, 2000. (art work and editing for book chapter)

Keefer L, Fitzhugh A. figures for patent application.

Liang R-T, Igarashi H, Tsuzuki T, Nakabeppu Y, Sekiguchi M, Kasprzak KS, Shiao Y-H. Presence of potential nickel-responsive element(s) in the mouse TH1 promoter. Ann. Clin. Lab. Sci. 31:9d1098, 2001. (art work)

MacDonald CJ, Ciolino H, Yeh GC. Dibenzoylmethane modulates aryl hydrocarbon receptor function and expression of cytochromes P450 1A1, 11A2, and 1B1. Cancer Res. 61:2919-2924, 2001. (art work)

Saavedra JE, Srinivasan A, Bonifant CL, Chu J, Shanklin AP, Flippen-Anderson JL, Rice WG, Turpin JA, Davies KM, Keefer LK. The secondary amine/nitric oxide complex ion R2N[N(O)NO]- as nucleophile and leaving group in SNAr reactions. J. Org. Chem. 66:3090-3098, 2001. (chemical structures)

Salcedo R, Resau JH, Halverson D, Hudson EA, Dambach M, Powell D, Wasserman K, and Oppenheim JJ. Differential expression and responsiveness of chemokine receptors (CXCr1-3) by human microvascular endothelial cells and umbilical vein endothelial cells. FASEB J. 14:20065-2974, 2000. (cover art)

Yen C-H, Yang Y-C, Ruscetti SK, Kirken RA, Dai R, Li C-C. Involvement of the ubiquitin-proteosome pathway in the degradation of nontyrosine kinase-type cytokine receptors of IL-9, IL-2, and erythropoietin. J. Immunol. 165:65372-6380, 2000. (composites)

Presentations:

Frederickson RF, Kane AR. Certificate of Merit for poster designed for Dr. Lidia Hernandez, et al., CDBL, NCI; presented at the Annual Meeting of the BioCommunications Association. Dallas, Texas, June 2001.

Repository Services

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Research Technology Program, SAIC-Frederick, Inc.

Web page: http://web.ncifcrf.gov/rtp/repos-serv.stm

Convenient, safe, cost-effective storage of research specimens

Mission: The NCI-Frederick Repository Service provides the NCI and other investigators with a convenient, safe, and cost-effective way to store research specimens.

Expertise: The NCI-Frederick Repository Service provides cryogenic services, including low-temperature storage from +4°C to -196°C, controlled-rate freezing, and a computerized inventory tracking system.

Established Technologies:

Features and Equipment:

- Programmable (CryoMed) Controlled Rate Sample Freezing
- Backup Generator Services for All Mechanical Freezers
- 24/7 Physical Security and Equipment Surveillance

Freezers:

- Sensor Controlled Vapor Phase Storage, Liquid Nitrogen
- Sensor Controlled Liquid Phase Storage (e.g., embryos), Liquid Nitrogen
- –70°C
- –20°C
- +4°C
- Ambient

Inventory Systems:

- BioSpecimen Inventory (BSI) – DCEG
- Natural Products Repository System (NPRS) – DCTD
- CenRep (Complete System/Natural Session)

New Technologies Under Development:

- Downloadable sample records
- Electronic Input Form preparation

Access

Open

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Major Equipment:

–70°C and -80°C chest and upright freezers

Liquid nitrogen freezers

–20°C Walk-In cold rooms

4°C Walk-In cold rooms

CryoMed controlled rate freezing unit

A

B

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